This raised new questions: what was the nature of the interactions with FcγRIIB, and which cells were involved?

Lazarus' group then dissected the mechanism of action of IVIg through an elegant series of preclinical studies, including the current report. They confirmed that FcγRIIB is a prerequisite for the IVIg effect in the passive-sensitization ITP model. Surprisingly, signal transduction molecules (eg, SHIP) did not abrogate the IVIg effect when mutated. Recently, they showed that dendritic cells preincubated with IVIg in vitro could recapitulate the therapeutic effect of IVIg and ameliorate murine ITP. These IVIg-primed leukocytes had effect only when the recipient mouse expressed FcγRIIB, whereas FcγRIIB was not required on the "initiator" dendritic cells; thus, FcγRIIB is not the direct target of IVIg, but is important downstream.[4] Moreover, the effect of IVIg was a consequence of IVIg driving activating FcγR chain signaling, although the FcγR itself was not required.

Nearly simultaneously, Ravetch's group demonstrated that the FcγRIIB-mediated effect of IVIg depended entirely on sialylated IVIg. Sialylated-enriched IVIg was 10-fold more efficacious than normal IVIg .[5] As non-sialylated IgG is unable to bind FcγR, this is consistent with Lazarus' demonstration of FcγR signaling on dendritic cells as necessary for the IVIg effect.

The current study explores the role of multiple cytokines and complement pathway components in IVIg-induced dendritic cell FcγR signaling activation. None appears to influence the IVIg effect, including, specifically, interleukin-1 receptor antagonist (IL-1Ra).

Lazarus and colleagues' (and Ravetch and colleagues') studies lay the foundation for development of modified IVIg or other novel agents to replace IVIg. One important caveat is that in human ITP, cytokines triggered by infusion of IVIg may affect secretion of antiplatelet antibody or, via FcRn interaction, accelerate its elimination.[6] Furthermore, cytokine modulation may be more important in the later phases of the therapeutic response, and these effects are not captured in the acute murine disease model.

Unraveling the mechanism of the effect of IVIg in autoimmune disease, in particular on antibody-mediated platelet clearance, promises to lead to further therapeutic advances in the near future.

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IMMUNOBIOLOGY

Comment on Heemskerk et al, page 235

Monogamy and polygamy in T-cell receptor (TCR) chain association

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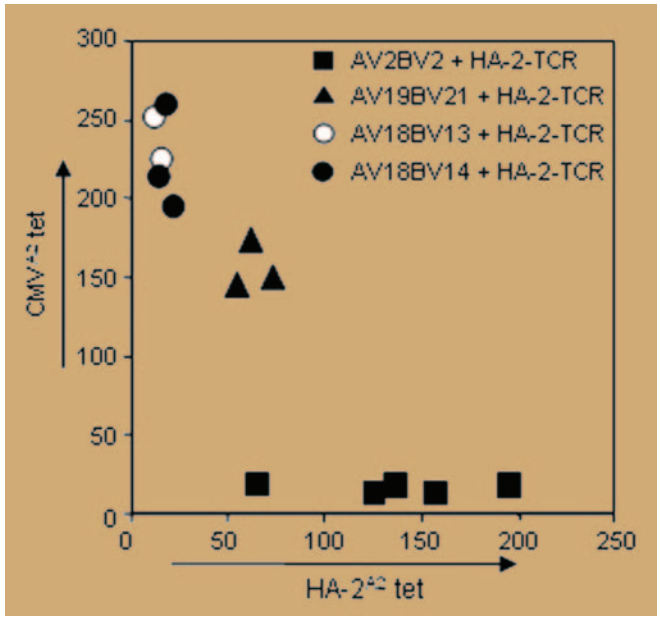
In this issue of *Blood*, Heemskerk and colleagues provide new insights into TCR chain pairing that will aid in the development of therapeutic T cells with new antigen specificities.

T cells once were thought to have a single-antigen specificity encoded by a unique T-cell receptor (TCR). However, many naturally circulating T cells are not allelically excluded at their TCRα locus.[1] When both α alleles functionally rearrange, T cells can express 2 different αβ receptor heterodimers. Dual-receptor expression may allow T cells expressing autoreactive receptors to avoid negative selection in the thymus, and may play a role in autoimmune diseases.[2]

The problem of polygamous pairing between TCRα and β chains may become even more complex in adoptive immunotherapy systems. Cells that target a specific antigen can be sparse, with natural frequencies of 1 in 10 000 or less. Immune tolerance may further leave specificity holes in the T-cell repertoire.[3] Acquiring adequate numbers of T cells specific for a desired antigen for immunotherapy is



Expression of a transgenic HA-2 TCR in 4 CMV-specific T-cell clones with different endogenous TCRα and β chains measured by antigen-tetramer staining. Levels of expression of endogenous versus transgenic TCRs vary with each clone. See the complete figure in the article on page 235.

thus a significant challenge. Transgenically introducing specific TCRs into T cells for adoptive immunotherapy is one solution.[4] Introduced TCRs can be derived from endogenous receptors or engineered to provide specificities or affinities not naturally present. Using retroviral vectors, it is possible to routinely transduce 15% to 50% of T lymphocytes, permitting the rapid generation of specific T cells. Indeed, the clinical potential of immunotherapy using T cells expressing introduced TCRs has recently been illustrated in patients with melanoma.[5] However, introducing an additional α and β chain into a T cell also increases the number of TCRs it can express. Transduced T cells with a single endogenous α chain can express 4 different receptors; T cells with 2 endogenous α chains can express up to 6. The impact of these additional receptors is unknown and a concern.

Heemskerk and colleagues have studied this by looking at chain pairing between a menagerie of introduced minor histocompatibility antigen (HA)–specific receptors and endogenous TCRs. They show that TCR pairing does not occur through random association, but rather is guided by intrinsic properties of the TCR chains. Two effects are apparent. First, some TCRα and β pairs associate particularly well and limit the formation of chimeric receptors in which introduced and endogenous receptor chains pair. This is an important finding that suggests it may be possible to identify TCRs less likely to form polygamous associations with endogenous receptors. Second, receptors compete for cell-surface expression, potentially through competition for the signaling CD3 chains of the TCR; some receptor heterodimers are better at this than others.

These results provide a starting point for analyzing how receptor pairing affects TCR cell surface expression. The implication is that TCRs used for adoptive immunotherapy must be assessed not only for their antigen specificity but also for their associative properties. Identifying optimal TCR remains empiric. However, a basis now exists for studying the biologic properties that govern TCR competition for association and for expression. Mutational and structural analyses of TCRs using systems like that presented by Heemskerk and colleagues can provide a better understanding of these biologic properties. This may permit the development of improved TCRs for use in adoptive immunotherapy.

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NEOPLASIA

Comment on Mestre-Escorihuela et al, page 271

Genomic loss is our gain

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Identification of biallelic chromosomal deletions in non-Hodgkin lymphoma cell lines coupled with gene expression profiling identifies novel tumor suppressor genes and potential therapeutic targets.

Chromosome imbalances occur frequently in B-cell non-Hodgkin lymphomas (B-NHLs), and likely contribute to pathogenesis via amplification of proto-oncogenes and deletion of tumor suppressor genes. Array-based comparative genomic hybridization (CGH) and high-density single-nucleotide polymorphism (SNP) arrays are sensitive genome-wide techniques to detect net gains and losses of genetic material. While application of these technologies has identified select imbalances with biologic and prognostic relevence,[1] the role of most of these imbalances remains poorly elucidated. Use of array-based CGH or high-density SNP array in concert with gene expression profiling allows focused investigation of genes within the imbalanced locus.

In this issue of *Blood*, Mestre-Escorihuela and colleagues report on the integrative use of array-based CGH and transcriptional profiling to identify novel tumor suppressor genes in a diverse set of B-NHL cell lines. Candidate genes are identified based on biallelic deletion coupled with decreased gene expression. Their analysis is then expanded to the broader set of B-NHLs to identify alternate mechanisms of tumor suppressor silencing.

Homozygous deletion of 16p13.13 is identified in a primary mediastinal B-cell lymphoma (PMBCL) line, with associated null expression of the gene *LITAF*. *LITAF* has not been well validated as a tumor suppressor, though it is a p*53*-inducible gene that may play a role in p*53*-mediated apoptosis.[2] In evaluating other cell lines, the authors find decreased *LITAF* expression in multiple diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL) resulting from promoter methylation. Given the germinal center derivation of these lymphomas, *LITAF* silencing is notable because it is repressed by *BCL6*,[3] a gene critical to the germinal center reaction, and the target of the most common recurring chromosome translocation in DLBCL. Though BCL6 directly suppresses expression of *p53*,[4] *BCL6*-mediated repression of *LITAF* appears to occur independently of TP53.[3] The authors accordingly demonstrate that BCL6 and LITAF are inversely expressed at the protein level, and that treatment with the hypomethylating agent, 5-azacytidine, restores LITAF expression. They do not report, however, whether re-expression affects cell viability. Such information would help tease out the significance of this particular gene in context of the protean downstream effects of *BCL6*.

The proapoptotic *BCL2* family member, *BIM*, is identified at a locus of homozygous deletion in mantle cell lymphoma (MCL), while decreased expression in BL and PMBCL cell lines is found secondary to promoter methylation. As with the epigenetic silencing of *LITAF* in DLBCL, 5-azacytidine restores BIM expression, though whether this results in apoptosis is again unclear. Another proapoptotic *BCL2* family member, *NOXA*, is silenced via homozygous deletion in a BL cell line, but also manifests decreased protein expression in additional cell lines due to a combination of heterozygous deletions with inactivating mutations of the remaining allele or promoter.