Insight *versus* Quagmire with Compound HIV Transgenics

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"*Why don't you mate mouse X with mouse Y and see what happens?*" How many times has one heard that bit of advice? For the beleaguered postdoc assigned to this experiment, the undoubted conclusion will be: Talk is cheap. Actually completing this experiment is expensive, labor-intensive, and time-consuming. The popularity of transgenic cross-breeding studies stems from the wide availability of numerous existing transgenic lines, but mostly, it is the lure of the definitive *in vivo* experiment. On the one hand, most definitive experiments usually are difficult and time-consuming. On the other hand, many investigators would argue that transgenic cross-breeding studies, whether it be by folly, naiveté, or bad luck, can be inadequately performed, making statistically significant data collection and interpretation of results challenging. With regard to the study of HIV-associated nephropathy (HIVAN), transgenic studies have been useful in illuminating aspects of pathogenesis; however, the literature is laden with countervailing reports from independent laboratories,[1] leaving both readers and investigators struggling to find a consensus.

The report by Feng *et al.*[2] in this issue of *JASN* was an opportunity to test definitively *in vivo* the well-developed hypothesis that HIVAN is caused by a virus-induced activation of the transcription factor STAT3. Current evidence suggests HIVAN is caused by HIV-1 infection of renal cells, and extensive *in vitro* work links several of the viral proteins, such as Nef and Vpr, with dysregulation of key host cell functions, subsequently inducing pathology. Feng *et al.* chose to validate their *in vitro* observations on STAT3-mediated pathogenesis by creating a compound transgenic mouse through cross-breeding two existing transgenic lines; therefore, this study began with a clear and directly testable hypothesis: Elimination of STAT3 signaling should prevent development of HIVAN. Unfortunately, the most direct strategy of cross-breeding a STAT3-null mouse with an HIV-1–transgenic mouse was not feasible, because STAT3-null mice are embryonic lethal. In addition, the HIV-1–transgenic mouse model used here, Tg26, is maintained as a heterozygote because homozygous animals do not survive to reproductive age. An additional issue with Tg26 mice is their dependence on a specific mouse genetic background, FVB/N, for disease manifestation,[3] a strain rarely used to create null mice.

To the authors' credit, they devise a clever strategy to deal with these challenging pragmatic issues. First, backcrossing STAT3 mice onto a disease-susceptible FVB/N background easily solved the genetic issue. This is easy in concept but expensive and time-consuming. Backcrossing to migrate transgenes onto new genetic strains is 10 generations (*i.e.* making a congenic, with one mouse generation is approximately 3 mo); here, the authors took the backcrossing to six generations, reducing the C57Bl/6 background effects to <2%. Second is the issue of how to test the hypothesis without generating STAT3-null mice. The authors formulated a complicated breeding scheme using three transgenic lines: Heterozygous STAT3-null mice ($STAT3^{+/-}$); homozygous mice harboring a serine-to-alanine point mutation in STAT3, decreasing its functional activity by 50% ($STAT3^{SA/SA}$); and heterozygous Tg26 mice. After much cross-breeding and genotyping, the authors were able to create two types of mice for comparison: Tg26 heterozygous mice with 25% STAT3 activity ($Tg26^{SA/-}$) and Tg26 heterozygous mice with 75% STAT3 activity ($Tg26^{SA/+}$). This new strategy is based on previous observations that <50% STAT3 activity is insufficient for normal, healthy mice,[4,5] so the 75% STAT3 mice are "normal" controls for the "abnormal" 25% STAT3 mice.

Did the authors succeed in definitively testing their hypothesis? On the surface, yes; Tg26 mice with 25% STAT3 had attenuated kidney disease as compared with Tg26 mice with 75% STAT3 activity. An in-depth analysis of their data, however, has left unanswered or raised new questions in deciphering the mechanism of HIVAN pathogenesis.

One confounding issue with the study is that STAT3 null, $STAT3^{SA}$ mutant, and the HIV-1 transgenes all are expressed in many other somatic cells, not just the kidney. Of significance is the defective thymocyte survival in $STAT3^{SA/-}$ null mice, resulting in reduced thymocyte numbers.[4] To verify this phenotype does not influence the development of kidney disease in the compound transgenics, the authors undertook a careful analysis of the major immune cell types in all lymphoid compartments and found no differences among the normal, Tg26, and $Tg26^{SA/-}$ mice. Although supportive of their study's conclusions, this is an unexpected result considering the known phenotype in $STAT3^{SA/-}$ mice and, even more intriguing, because many of the HIV-1–transgenic mouse and rat models have perturbations in T cell popula-

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tions as a result of the widely known function of the viral protein Nef in downregulating cell surface receptors such as CD4 on T cells.

This issue with Nef function in renal cells *versus* leukocytes is of central importance to the hypothesis being tested, because this group previously showed that Nef causes STAT3 dysregulation in podocytes. The authors speculate this failure of Tg26 mice to recreate the widely known Nef-related CD4 downregulation is attributable to expression levels, suggesting there is sufficient Nef to activate STAT3 in the kidney but insufficient Nef to downregulate CD4 in T cells; however, a previous comparison of expression levels between kidney and lymphoid organs in Tg26 heterozygous mice is similar.[6] Moreover, the protein trafficking functions of Nef (CD4 downregulation) are separable from its function in mediating signal transduction events such as STAT3 activation, and ongoing transgenic work by the Jolicoeur laboratory is segregating the renal disease–causing effects of Nef through leukocytes *versus* renal cells[7,8]; however, this segregation is not attributable to levels of transgene expression. Nef has a dauntingly complex array of functions in host cells,[9] and exactly how Nef orchestrates pathogenesis in rodent models, let alone humans, is far from established definitively.

As Feng *et al.* demonstrate, creating compound transgenic mice is a sophisticated genetic approach to testing developmental and pathogenesis paradigms *in vivo* but with equally sophisticated challenges in design and execution. The strongest conclusion from this study is the overall role of STAT3 in the pathogenesis of HIVAN; however, there are issues in definitively attributing this to Nef alone in renal cells—the basis of their hypothesis—because Tg26 mice express Nef and many other HIV-1 proteins in other cell types. In light of their observations, the authors propose STAT3 should be a druggable target for HIVAN. Small molecule inhibitors for STAT3 are currently being development for cancer therapy and seem to have both antiproliferative and immunomodulatory properties.[10] Thus, this is a logical next step in which the Tg26 mouse will be a good small animal model for testing, although the specifics of drug action, such as cell targets, would remain unclear.

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DISCLOSURES

None.

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See related article, "Reduction of Stat3 Activity Attenuates HIV-Induced Kidney Injury," on pages 2138–2146.

Surprising Results following Conditional Podocyte Inactivation

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The genetic contribution to steroid-resistant nephrotic syndrome and related disorders of the podocyte has been widely appreciated only in the past decade. In fact, for at least 50 yr, nephrosis has been observed occasionally in multiple members of the same family. In a 1957 study, Farquhar *et al.*[1] performed

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