Helen E. Thomas,[1,2] Thomas C. Brodnicki,[1,2] and Thomas W.H. Kay[1,2]

How to Make Mice Tell the Truth

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Animal models have provided significant mechanistic insights into both physiology and disease. The NOD mouse, first identified and bred in Japan in the 1970s, is no exception. Studies in these mice have contributed to basic knowledge of the immune system, including costimulation, regulatory T cells, and MHC structure and function. NOD mice have also been informative about mechanisms that result in autoimmune diabetes, although it is unclear how these specifically contribute to disease pathogenesis in an individual human patient (1). Predisposing MHC genes, along with autoimmune reactions to insulin and its precursor proinsulin, are important in both mouse and human disease (2–6). Less clear is the value of animal models as part of the preclinical development of new treatments for disease. This has been particularly questioned not only for neurological diseases but also for type 1 diabetes (7). This uncertainty stems from many factors: the idea that inbred strains are a genetically homogeneous "n of 1" experiment compared with outbred humans; the many well-documented differences in the immune systems of the two species; and, most relevant to the article by Gill et al. (8), issues around the reliability, robustness, and reproducibility of data produced in trials of therapy in mice. These concerns have undermined the value of mouse studies and led to the notion that "mice lie," and it is at least in part suboptimal design and execution of studies that have led to outcomes that cannot be translated to humans.

To address these concerns, a consortium was formed by JDRF and the Immune Tolerance Network to carry out high-quality, multicenter trials using many of the techniques and disciplines of human clinical research. The first of these trials tested the combination of anti-CD3 and interleukin-1 (IL-1) blockade in NOD mice with newly diagnosed diabetes (8), which was found previously by one member of the consortium to be significantly more efficacious than anti-CD3 alone (9). Combination therapies such as this are in the process of being tested in human subjects to preserve β-cell function after diagnosis of diabetes (10). This time period, after diagnosis, is challenging for diabetes reversal, and recent commentaries emphasize that high-risk subjects prior to diagnosis of diabetes already have disease suitable for more aggressive treatment (11). Although the study by Gill et al. (8) used newly diagnosed NOD mice, the appropriate mouse cohort to study potential new therapies remains a matter for debate. Apart from being multicenter (a study type used in clinical research to recruit sufficient numbers but here used to compare centers and reduce variability), this study also used standard operating procedures (SOPs), standardized reagents, and randomization to active treatment and placebo. The purpose of this was to determine whether results varied significantly within and between institutions or were consistent.

Despite the provision of standardized reagents and SOPs, as well as the lack of any incentive to show positive results to achieve publication, the results were divergent between the individual sites (8). Two sites were able to clearly achieve reversal of diabetes with anti-CD3, one site achieved reversal to some extent, and one site achieved minimal reversal. None of the sites were able to exactly reproduce the data from the previous study showing the benefit of IL-1 blockade combined with anti-CD3 (9), although one site found a modest effect of IL-1 blockade when it was added sequentially rather than at the time that anti-CD3 was given. There were differences between the protocol in the pilot study and the multicenter study, with a smaller dose of anti-CD3 and initial treatment with insulin used in the latter. The pooled data from the centers clearly showed an effect of anti-CD3 to reverse diabetes, despite the lower dose, and no additional effect of IL-1 blockade either with anti–IL-1β or with IL-1trap. IL-1 blockade on its own had no effect, which is consistent with the diabetes incidence observed in our study of NOD mice lacking the IL-1 receptor (12).

IL-1 is a plausible target in many immune-based diseases. Effective and relatively safe inhibitors are also available and

[1]St Vincent's Institute, Fitzroy, Victoria, Australia
[2]Department of Medicine, St Vincent's Hospital, The University of Melbourne, Fitzroy, Victoria, Australia
Corresponding author: Helen E. Thomas, hthomas@svi.edu.au.

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have been licensed for use in rheumatoid arthritis and other diseases (13). Nonetheless, there is relatively little direct evidence for the impact of IL-1 in type 1 diabetes. For example, IL-1–responsive gene expression has not been clearly identified in islets from humans or mice with autoimmune diabetes, whereas interferon signatures have been identified in both (14–17). One suggestion of a role for IL-1 comes from an indirect assessment of cytokines in which plasma is incubated with peripheral blood mononuclear cells from an unrelated individual, and gene expression is measured (18). Although an interesting development, this novel assay requires further validation. Moreover, it has been shown that IL-1 antagonists alone do not preserve β-cell function in type 1 diabetes (19). Therefore, the negative outcome of the current study is perhaps not unexpected and provides further evidence for a modest role for IL-1 in diabetes in NOD mice.

Has this valuable study raised the bar for all studies in NOD mice? The answer to this is certainly yes, in that a high degree of rigor should be routine in testing new therapies in preclinical models. The criteria that need to be considered include those in Table 1. However, it is unlikely that multicenter trials will entirely replace single-center studies. Single-center studies will continue to be useful, especially if well designed, and are likely to be cheaper for initial tests compared with multicenter studies that require substantial support from funding agencies. Following a positive result, additional steps, such as the consortium formed for the current study, could be used before new treatment regimens proceed to human clinical trials.

Table 1—Criteria for testing a therapeutic treatment in NOD mice

- Ensure NOD females have a high incidence of diabetes (e.g., ≥70% diabetes incidence by 300 days of age) in the chosen animal facility if a preventative study is being performed
- Establish an SOP and fixed study entry criteria for the treatment regimen
- Perform a power calculation to determine the minimum cohort size required to detect an effect of the desired size
- Determine what controls are required to evaluate treatment efficacy
- Monitor diabetes incidence in controls contemporaneously with treated mice
- Ensure cohorts are tested in a timely fashion to avoid variable diabetes incidences over a long period
- Use pharmaceutical-grade reagents when possible
- Generate sufficient reagent stocks for treatment of all mice to minimize effects due to different reagent preparations
- Randomize treatment within age-matched littermates
- Have the same experienced personnel perform the treatment and monitoring protocols for all mice

Ideally, these criteria should be identical for single-lab and multicenter trials. However, it is well recognized that initial testing of a proposed treatment by a single lab may not be optimal because of various factors (e.g., the most effective treatment protocol is not obvious, pharmaceutical-grade reagents are not readily available, or technical proficiency for performing treatment and monitoring protocols may be limited). This should not preclude the publishing of such studies as long as the caveats for the results are clearly described by the investigators. Indeed, the advantage of the NOD mouse model is that a promising treatment protocol can be further tested and potentially improved upon by other groups.

A successful outcome of therapy is more likely in mice because experiments can be designed to increase the prospect of a positive outcome. For example, in the current study, screening was able to identify diabetic mice very soon after they became diabetic, which is currently very difficult to do in humans. Treatment is sometimes begun even before autoimmunity, in the first weeks of life; again, this approach is currently not possible in humans. If a therapy is unsuccessful at this multicenter-trial step, as is the case for anti-CD3 and IL-1 antagonism, should plans to test it in humans be shelved? There is a strong case that it will be unsuccessful in humans. However, the mechanisms of human and NOD autoimmune diabetes are not identical; if other data support a therapy being tested in humans, it may be reasonable to proceed, especially because humans are heterogeneous. Conversely, a successful outcome in a robustly conducted trial with NOD mice would support consideration of a human clinical trial but not guarantee its result.

The NOD mouse strain is arguably the best preclinical animal model we have for type 1 diabetes. When a treatment succeeds or fails in NOD mice, there are a variety of powerful mechanistic studies that can explain why. NOD colonies are also maintained around the world so that initial studies can be replicated and improved upon by other groups. The study by Gill et al. provides an excellent example of how this can be done in a rigorous manner and shows that NOD mouse studies, if applied well, have an important role in the development of new treatments for type 1 diabetes.

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