

# An Apparent Role for *Alox15* in the Pathogenesis of Diabetes in the NOD Mouse

## Parsing the Supporting Genetic Data

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**P**rogenitors of the nonobese diabetic mouse strain (NOD/Shi) arose spontaneously in a colony at the Shionogi Research Laboratories in Aburahi, Japan in the 1970s. The strain has been used extensively in efforts to elucidate the pathogenesis of type 1 diabetes in humans. Both the mouse model and the human disease are characterized by the appearance of autoreactive T-cells targeting pancreatic islet antigens, the elaboration of anti-insulin autoantibodies, and the development of a  $\beta$ -cell-toxic inflammatory cellular infiltrate within the islets leading to insulin depletion and hyperglycemia. In both NOD mice and human type 1 diabetes, the breakdown of self-tolerance to  $\beta$ -cells is under polygenic control of major histocompatibility complex (MHC) class II alleles as well as non-MHC loci. An important difference between the mouse model and the human disease is that in NOD mice but not human type 1 diabetes, female subjects have a higher incidence of diabetes (75–100% by 30 weeks) than males (30–60%) (1). Because the development of diabetes in NOD mice depends on interacting genetic and environmental factors comparable with those interacting in human type 1 diabetes, genes that modify the NOD phenotype deserve close attention. The article by McDuffie et al. (2) in this issue identifies such a candidate molecule.

Previous studies elucidating the critical steps in the development of autoimmune  $\beta$ -cell destruction in NOD mice have suggested several points of pathogenic relevance.  $\beta$ -Cell destruction in NOD mice is dependent on the production and unrestrained activation of autoreactive T-cells. Antibody-mediated elimination of T-cells and interventions that promote T-cell tolerance, such as the production of regulatory T-cells, effectively inhibit development of diabetes in NOD mice (3). To a limited extent, this approach has shown promise in humans with type 1 diabetes (4). In addition, inhibiting the function of the MHC class II-expressing antigen presenting cells (e.g., macrophages and dendritic cells) known to infiltrate islets early in the development of islet inflammation may prevent

stimulation and proliferation of autoreactive T-cells and thereby the development of autoimmune diabetes (5).

McDuffie et al. (2) implicate a 12/15-lipoxygenase encoded by *Alox15* in the development of autoimmune diabetes in NOD mice. They identified *Alox15* as a candidate gene for modifying diabetes susceptibility in NOD mice because it lies within an ~20-Mb genetic interval containing the NOD diabetes susceptibility locus *Idd4.1* (6). The lipoxygenase encoded by *Alox15* is highly expressed in both macrophages and  $\beta$ -cells, and it oxidizes the polyunsaturated fatty acids arachidonic acid and linoleic acid, creating unstable lipids with potent cytotoxic and proinflammatory activity. In previous work, these investigators showed that *Alox15* deficiency confers protection from diabetes in C57BL/6J mice injected with the  $\beta$ -cell toxin streptozotocin. The mechanism of diabetes protection in the streptozotocin-induced diabetes model appears to involve decreased cytokine-mediated  $\beta$ -cell toxicity and decreased macrophage production of nitric oxide (7). Subsequent work has shown that 12/15-lipoxygenase promotes monocyte/endothelial cell interactions in diabetic mice, and macrophage secretion of interleukin-12, an important promoter of T-cell activation and production of inflammatory cytokines such as  $\gamma$ -interferon that drive the progression of autoimmune diabetes (8,9).

To test the hypothesis that *Alox15* deficiency confers protection from autoimmune diabetes, McDuffie et al. used a genetics-based strategy and introgressed a region of chromosome 11 containing the *Alox15*<sup>*tm1fun*</sup>-null mutation into NOD mice. Their resulting NOD congenic line (NOD-B6.129S2-*Alox15*<sup>*tm1fun*</sup>) is homozygous for the *Alox15* mutation and the ~10-Mb B6.129S2-derived genetic fragment surrounding it, and that traveled in with the null *Alox15* allele during the introgression. These congenic mice are substantially protected from the development of diabetes compared with the original NOD strain that segregates for a functional *Alox15* allele. The *Alox15*-null NOD congenics also display decreased early macrophage recruitment to islets, decreased T-cell infiltration into islets, and increased numbers of regulatory T-cells, suggesting that *Alox15* deficiency prevents the development of diabetes by interrupting the development of autoimmunity (2). If these data prove to be relevant to the pathogenesis of type 1 diabetes in humans, then novel therapeutic interventions may be developed based on impairing production of lipids that are proinflammatory and toxic to  $\beta$ -cells.

However, McDuffie et al. also correctly emphasize the limitations inherent in their “candidate gene”/congenic approach to identifying gene(s) underlying the phenotype of the *Idd4* congenic lines. In the *Alox15*-deficient NOD congenic line, the 10-Mb B6.129S2-derived fragment

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MHC, major histocompatibility complex.

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linked to *Alox15<sup>tm1fun</sup>* replaces almost one-half of the ~20-Mb NOD-derived interval known to contain *Idd4.1*. Therefore, in addition to the null *Alox15<sup>tm1fun</sup>* allele that was intentionally introgressed, the NOD-B6.129S2-*Alox15<sup>tm1fun</sup>* mouse line has many other DNA sequence differences (vs. those of NOD) throughout this portion of the *Idd4.1* interval. These differences cannot be discounted as plausible explanations for some, or possibly even all, of the observed phenotypes of the new congenic line. However, the earlier results in streptozotocin-injected C57BL/6J mice add support for a likely role of *Alox15* in these NOD mice.

The replaced B6.129S2 interval contains 317 genes including 5 that encode lipoxygenase enzymes in addition to *Alox15* and multiple other genes that encode known regulators of inflammation such as CD68 and CXCL-16. In addition, the interval contains the B6 allele of the gene *Trpv1*, which has been shown to mediate sensory neuron control of islet inflammation and development of autoimmune diabetes in NOD mice. The NOD allele of this gene contains two missense mutations altering highly conserved amino acids, and it is hypofunctional compared with the B6 allele (10). However, there is no reason to assume that possibly confounding sequence differences must lie within regions of protein encoding DNA. Although it is often difficult to predict the functional impact of noncoding sequence variants, it is estimated that 70% of highly evolutionarily conserved sequences do not encode proteins and that many of these sequences contain important determinants of gene expression and thereby modulate disease risk (11,12). Because quantitative trait locus mapping is inherently biased to find regions that have a strong influence on phenotype, these regions may contain more than one gene influencing the phenotype. Thus, the null *Alox15* allele may act in concert with other coding and noncoding diabetes-related sequence variants within *Idd4.1* to produce the protection from diabetes observed in NOD-B6.129S2-*Alox15<sup>tm1fun</sup>* mice.

Because NOD embryonic stem cells are not competent for homologous recombination and germline transmission of targeted genetic changes, genetic experiments in these animals usually employ the creation of congenic substrains, with, as noted, intervals of potentially confounding allelic variation from the donor strain flanking the gene of interest (13). To circumvent this problem, one option might be the use of short hairpin RNA-containing lentiviral vectors to infect NOD zygotes, thus creating NOD transgenics with knockdown of targeted genes (14). Also, mouse haplotype maps and direct sequence comparisons now enable detailed assessment of interstrain sequence variation relevant to potentially confounding genetic variation within congenic intervals (15,16). For example, a recent study by Yamanouchi et al. (17) showed that *Il2* haploinsufficiency was sufficient to increase diabetes susceptibility in an NOD substrain. However, the *Il2*-null allele was introgressed into NOD from a 129 background. To show that the 129 sequence flanking the *Il2*-null allele likely did not account for the phenotype, they cited a map of sequence variation between NOD and 129 that showed that the two species were identical by descent (0.68 single nucleotide polymorphisms per 10 Kb) throughout the relevant genetic interval.

The article by McDuffie et al. should spur efforts to further define the role of 12/15-lipoxygenase in development of type 1 diabetes. The discovery of a single

genetic change that has a major impact on diabetes susceptibility in NOD mice is potentially important. However these data should also be interpreted with caution because the mechanisms underlying the altered phenotypes of the NOD-B6.129S2-*Alox15<sup>tm1fun</sup>* mice may themselves be multigenic.

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