Diabetic Mouse Incorrectly Typed

In our report "Three recessive loci required for insulin-dependent diabetes in nonobese diabetic mice" (*1*), we outcrossed diabetes-susceptible strain NOD mice with diabetes-resistant strain NON, backcrossed the resistant F_1 hybrids to the susceptible NOD parental strain, and analyzed segregation of diabetogenic genes. We reported that 1 of 19 mice developing diabetes at first backcross apparently exhibited recombination within the major histocompatibility complex (MHC) on chromosome 17. Microcytotoxicity testing of splenic leukocytes with monoclonal antibodies and complement indicated homozygosity for the *H-2K*^{d} allele (NOD type), but expression of *I-E*^{k} (NON type). Since a recessive diabetogenic allele (*Idd-1*^{s}) tightly linked to MHC was segregating in this cross, the inference was that, if this gene were within MHC, it would lie proximal to the *I-E* locus. To confirm intra-MHC recombination, encoded samples of Bam HI–cut genomic DNA from the putative recombinant and the other diabetic segregants were analyzed by Southern blotting with the use of an *I-E*$_\beta$–specific probe. DNA from the putative recombinant mouse was identified as the only sample producing an "F_1-like" restriction fragment length polymorphism (RFLP).

On the basis of subsequent detailed molecular genetic analysis of DNA from this individual, we regret to report that the original recombinant designation was erroneous, apparently the result both of a false-positive microcytotoxicity assay and of a deviant Bam HI digestion of that particular DNA sample. We used subcloned probes recently described by Passmore *et al.* (*2*) and informative for RFLP distinguishing the NOD and NON parentals and F_1 to analyze *A*α (probe 2, Pvu II digest), *E*$_\beta$ (probe 3, Bam H1 digest), *E*$_{\beta 2}$ (probe 6, Xba I digest), and *E*α (probe 7, Taq I digest). Southern blots showed only the NOD parental restriction fragments at these four loci.

Nishimoto *et al.* (*3*) recently reported that transgenic mice expressing *I-E*^{d} on a segregating NOD genetic background were resistant to development of insulitis. This suggests that mutation in the *I-E* gene preventing expression of *I-E* gene product was itself the diabetogenic mutation. In favor of this hypothesis is our study, updated by the correction presented in this letter, showing that all 19 of the diabetic first backcross mice were NOD-like in nonexpression of an *I-E* gene product. However, in our study, MHC heterozygosity did not protect from insulitis, since variable numbers of islets with insulitis were recorded in 16 of 70 nondiabetic MHC heterozygotes studied at first backcross. In addition, 54 of these 70 heterozygotes exhibited leukocytic infiltrates in the pancreas. The potential protective role of *I-E* gene expression will best be assessed by injecting a functional *I-E* gene directly into NOD embryos and by analyzing the incidence of overt diabetes in aging transgenic and wild-type progeny.

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