

SUPPLEMENTAL MATERIAL

Bergman et al., <https://doi.org/10.1084/jem.20160471>

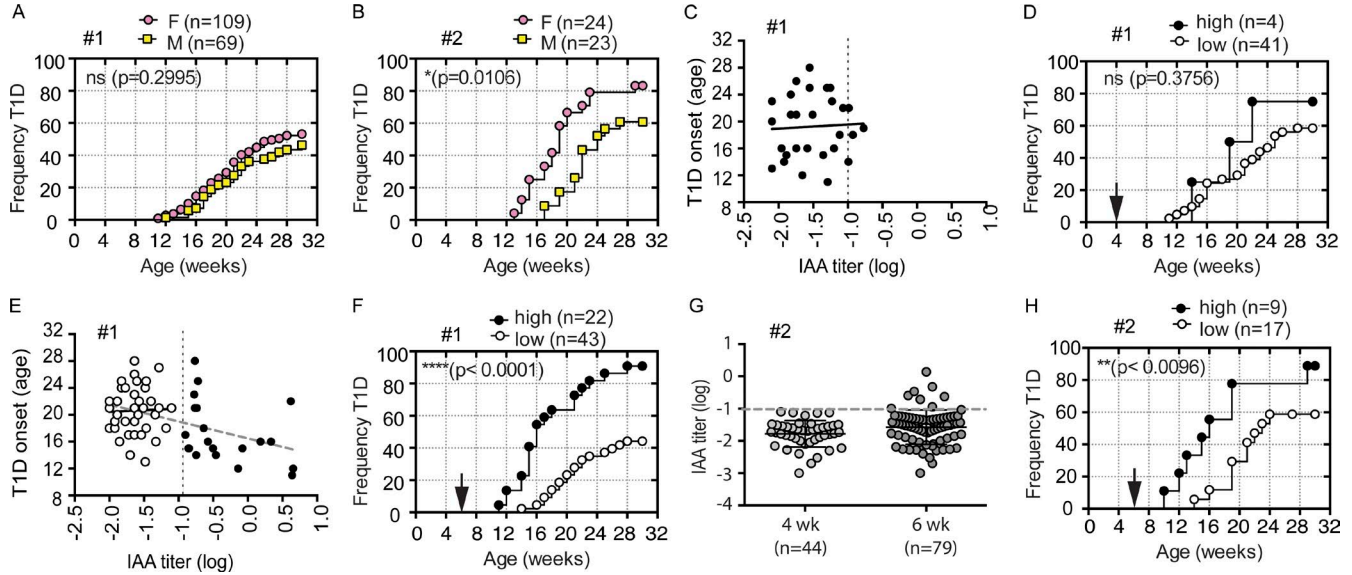


Figure S1. Age-dependent correlation between IAA titer and T1D onset and incidence in two NOD colonies. Glycemia was monitored weekly from 10 to 30 wk of age, and IAA titers were monitored as indicated. (A and B) Incidence and onset of hyperglycemia, as well as gender biases, differ between colonies #1 (A) and #2 (B). T1D incidence is presented in Kaplan-Meier survival plots, males ($n = 68$) and females ($n = 109$) of colony #1 (A) and males ($n = 23$) and females ($n = 24$) of colony #2 (B). Log-rank tests defined statistical differences for T1D incidence between males and females: not different for colony #1 ($P = 0.2995$), different for colony 2 ($P = 0.0106$). Pooled data from batches of 4–16 age-matched mice. (C and D) Serum IAA titers at 4 wk of age are not predictive of T1D onset or incidence in colony #1. Serum IAA titers were measured at 4 wk of age (4w-IAA) in 45 NOD females from colony #1. (C) Linear regression analysis of T1D onset as a function of 4w-IAA titer (plain line) shows no correlation ($r^2 = 0.0025$, $P = 0.8$, $1/\text{slope} = 1.6$). (D) T1D incidence in the same NOD females, now sorted as 4w-IAA^{high} ($n = 4$) or 4w-IAA^{low} ($n = 41$) according to a cut-off of 0.1 (defined below in E and represented by a vertical dotted line in C) are presented in a Kaplan-Meier survival plot. Log-rank tests showed no statistical differences ($P = 0.3756$). Pooled data from batches of three to nine age-matched mice. 4w-IAA^{low} mice in (D), $n = 41$, are different animals than in Fig. 1 A. (E and F) IAA titers measured at 6 wk of age predict onset and incidence in colony #1. (E) Serum IAA titers were measured at 6 wk of age in 65 females from colony #1. When considering all animals (white and black circles), linear regression analysis of T1D onset as a function of IAA titer measured at 6 wk of age (dashed gray line), IAA titer explains 20% of the variation in T1D onset ($r^2 = 0.1828$, $P = 0.0009$, $1/\text{slope} = -3971$), and the optimal threshold for T1D predictability was 0.1, identifying 31% of the mice as 6w-IAA^{high}. In agreement with this threshold, analysis of data limited to titers < 0.1 (white circles, $n = 37$, plain line) show no correlation ($r^2 = 0.00105$, $P = 0.8491$, $1/\text{slope} = 2.039$). (F) The same mice were sorted for IAA level at 6 wk of age using a cut-off of 0.1 (dotted black line in E), and T1D incidence plotted in a Kaplan-Meier survival plot ($n = 43$ for 6w-IAA^{low}, $n = 22$ for 6w-IAA^{high}). Log-rank tests defined statistical differences for T1D incidence ($P = 0.0001$). Selection for 6w-IAA^{low} brought diabetes incidence at 30 wk of age from 53% (A) to 43%. Pooled data from batches of 7–16 age-matched mice. 6w-IAA^{low} mice in E and F are different animals than in Fig. 1 (C and F). (G and H) IAA titers measured at 6 but not 4 wk of age predict disease onset and incidence in colony #2. (G) IAA titer in females from colony #2. 44 females were tested for serum IAA levels at 4 and, together with another 35 females ($n = 79$), at 6 wk of age. The 0.1 threshold (dashed line) identified 10% of the mice as 6w-IAA^{high} and none as 4w-IAA^{high}. (H) Glycemia was measured for 30 wk in all mice identified as 6w-IAA^{high} in G ($n = 9$, originating from eight different litters), and in 17 of those identified as 6w-IAA^{low}. Log-rank tests defined statistical differences for T1D incidence in Kaplan-Meier survival plots as $P = 0.0096$. Selection of 6w-IAA^{low} mice brought diabetes incidence at 30 wk of age from 83% to 60%.