Supporting Information

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SI Materials and Methods

Mice and Sera. The *Aire*-knockout mutation, originally generated on a mixed [B6 × 129/Sv] F2 genetic background, was genotyped as described (1), and was backcrossed to the NOD and BALB/c backgrounds for more than eight generations (2). B6.μMT−/− mice (3) were purchased from the Jackson Laboratory and were genotyped with the sense primer 5′-CCGTCTAGCTTGAGCTATTAGG-3′ and anti-sense primer 5′-GAAGAGGACGATGAAGGTGG-3′ for 35 cycles of a PCR with an annealing temperature of 55°C. They were either intercrossed with *Aire*+/− mice of the mixed B6 × 129 background, or were backcrossed to the NOD background for 12 generations, then intercrossed with NOD.*Aire*+/− mice. *Jh*-knockout mice on the BALB/c background were purchased from Taconic Farms and were genotyped for the deletion of the J region of the heavy chain with the sense primer 5′-CCAGGGACTCCACCAACACC-3′ and anti-sense primer 5′-GTAAGAATGGCCTCTCCAGG-3′, for 35 cycles of a PCR with an annealing temperature of 60°C. They were intercrossed with BALB/c.*Aire*+/− mice. NOD mice bearing the huCD20 transgene were typed with the sense primer 5′-TTGAGAGCAAAATGACAACACCCA-3′ and anti-sense primer 5′-AGGAAGCAGTGGAGGTGCCAAGAA-3′ for 35 cycles of a PCR with an annealing temperature of 60°C. They were intercrossed with NOD.*Aire*+/− mice. All mice were housed and bred under specific-pathogen-free conditions at the Harvard Medical School Center for Animal Resources and Comparative Medicine.

Flow Cytometry. Cell suspensions were teased from spleens and analyzed by flow cytometry after immunostaining with anti-CD19 (6D5), anti-CD45R (B220, RA3–6B2), anti-CD4$^+$, and anti-CD8$^+$ Abs, conjugated either with fluorescein isothiocyanate or phycoerythrin (Caltag).

Western Blotting. The protein was briefly heated at 96°C, resolved on a 10% curtain gel by SDS/PAGE, and transferred onto polyvinylidene fluoride membrane (Bio-Rad Laboratories). The membrane was blocked for 1 h in a 5% milk solution in Tris-buffered saline (TBS; 25 mM Tris, pH 7.6, and 150 mM NaCl) and was probed in a Protean II Multiscreen apparatus (Bio-Rad Laboratories) with mouse sera diluted 1:500 for 2 h at 4°C. The membrane was washed with TBST (TBS, 0.1% Tween-20), incubated with horseradish peroxidase-conjugated donkey anti-mouse IgG (1:3,000) (Jackson ImmunoResearch), and was revealed with SuperSignal chemiluminescent substrate reagents (Pierce) by autoradiography.

Serum IgG Measurement. Test sera were diluted at 1:1,000 and added in duplicate to the ELISA plates for 1 h at room temperature (RT). Uncaptured serum Ig was washed as above. Alkaline-phosphatase-conjugated goat anti-mouse IgG (Fc-specific) (Jackson ImmunoResearch) was added at a dilution of 1:2,000 at RT for 1 h; the antibody incubation was followed by another set of four washes. Bound Ig was detected by measuring the absorbtion at 405 nm of an alkaline phosphatase substrate, buffered with 9.6% diethanolamine (vol/vol H_2O) and 2 M $MgCl_2$, as recommended by the manufacturer (Sigma). Ig concentrations were calculated by relating absorptions of test samples to those of a standard curve.

1. Anderson MS, *et al.* (2002) Projection of an immunological self shadow within the thymus by the aire protein. *Science* 298:1395–1401.
2. Jiang W, *et al.* (2005) Modifier loci condition autoimmunity provoked by Aire deficiency. *J Exp Med* 202:805–815.
3. Kitamura D, Roes J, Kuhn R, Rajewsky K (1991) A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin mu chain gene. *Nature* 350:423–426.

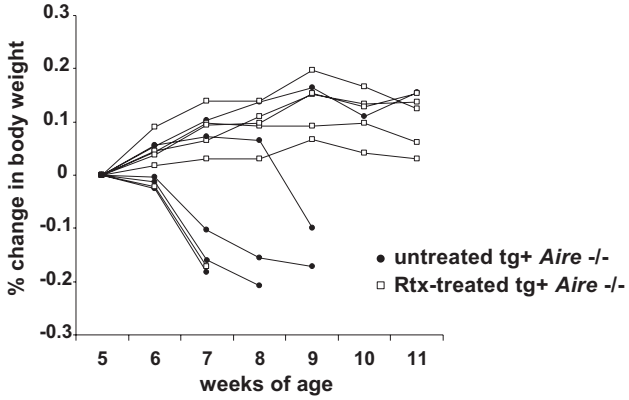

Fig. S1. Percent change in body weight in Rituximab-treated (●) and untreated (□) NOD.*Aire*−/− mice during a regimen initiated at 5 weeks of age.