Supplemental Data

Central Role of a Defective Interleukin-2 Production

in the Triggering of Islet Autoimmune Destruction

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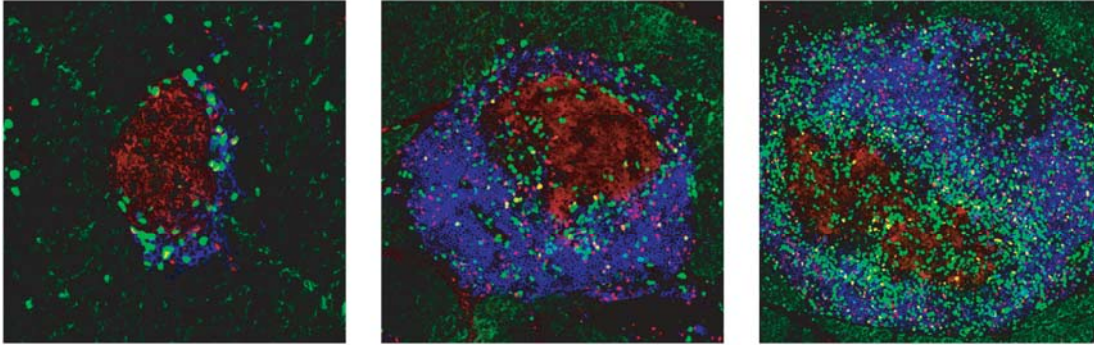

Figure S1. Immunofluorescent Analysis of Intra-Islet Infiltrates

Sections of pancreata from NOD of various ages and diabetes status were stained with anti-CD4 (blue), anti-Foxp3 (red, nuclear stain), anti-Ki76 (green), and insulin (red, diffused stain). Images of the stained samples were acquired on a confocal microscope and representative islets with mild (left), moderate (middle) and severe (right) inflammation are shown.
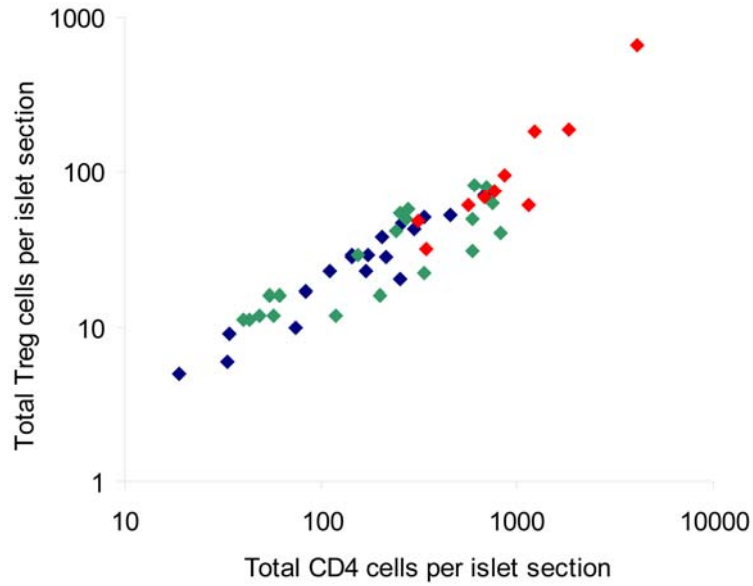
Figure S2. Absolute Numbers of Treg Cells as a Function of Total CD4 Cells per Islet Section.

Numbers of CD4^{+} and Foxp3^{+} cells in individual islets were quantified by manual counting of immunofluorescently stained frozen pancreatic sections. The total numbers of Foxp3^{+} Tregs in individual islet sections are plotted against the total number of CD4^{+} cells in the corresponding islet sections. Blue symbols represent islets from 6-8 week old NOD mice, green symbols are from 10-12 week old mice and red symbols are from mice with recent diabetes onset.

Figure S3. Absolute Numbers of Proliferating CD4 Teff Cells as a Function of Total CD4 Cells per Islet Section

Numbers of Ki67^{+} CD4^{+} Foxp3^{-} and CD4^{+}cells in individual islet sections were quantified by manual counting of immunofluorescently stained frozen pancreatic sections. The total numbers of Ki67^{+} CD4^{+} Foxp3^{-} in individual islet sections are plotted against the total number of CD4^{+} cells in the corresponding islet sections. Blue symbols represent islets from 6-8 week old NOD mice, green symbols are from 10-12 week old mice and red symbols are from mice with recent diabetes onset.
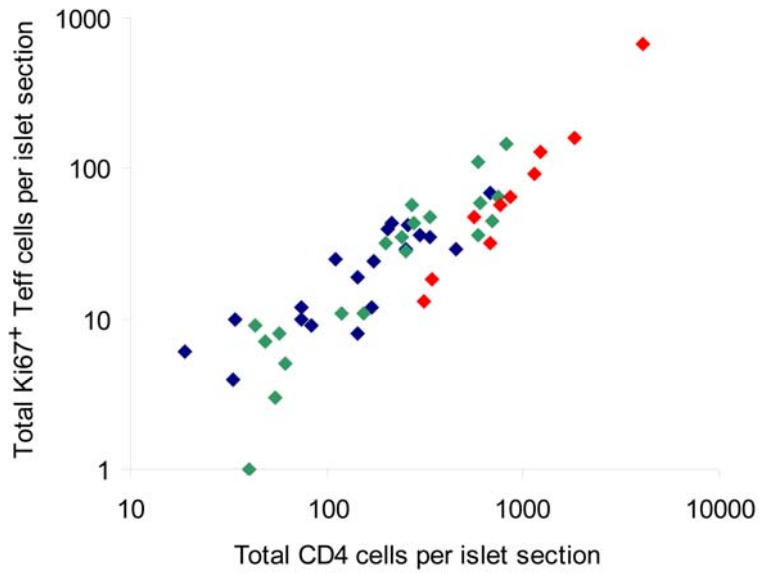
Figure S4. Absolute Numbers of Proliferating CD4 Treg Cells as a Function of Total CD4 Cells per Islet Section

Numbers of Ki67^{+} CD4^{+} Foxp3^{+} and CD4^{+}cells in individual islet sections were quantified by manual counting of immunofluorescently stained frozen pancreatic sections. The total numbers of Ki67^{+} CD4^{+} Foxp3^{+} in individual islet sections are plotted against the total number of CD4^{+} cells in the corresponding islet sections. Blue symbols represent islets from 6-8 week old NOD mice, green symbols are from 10-12 week old mice and red symbols are from mice with recent diabetes onset.
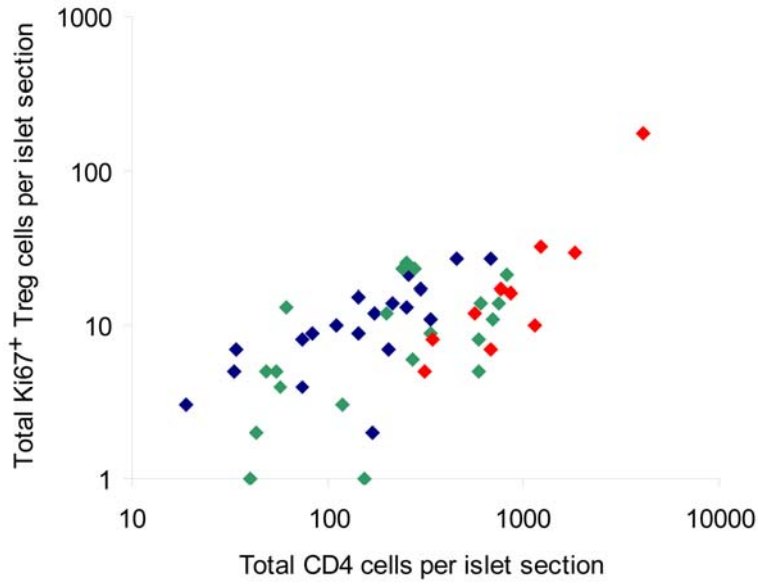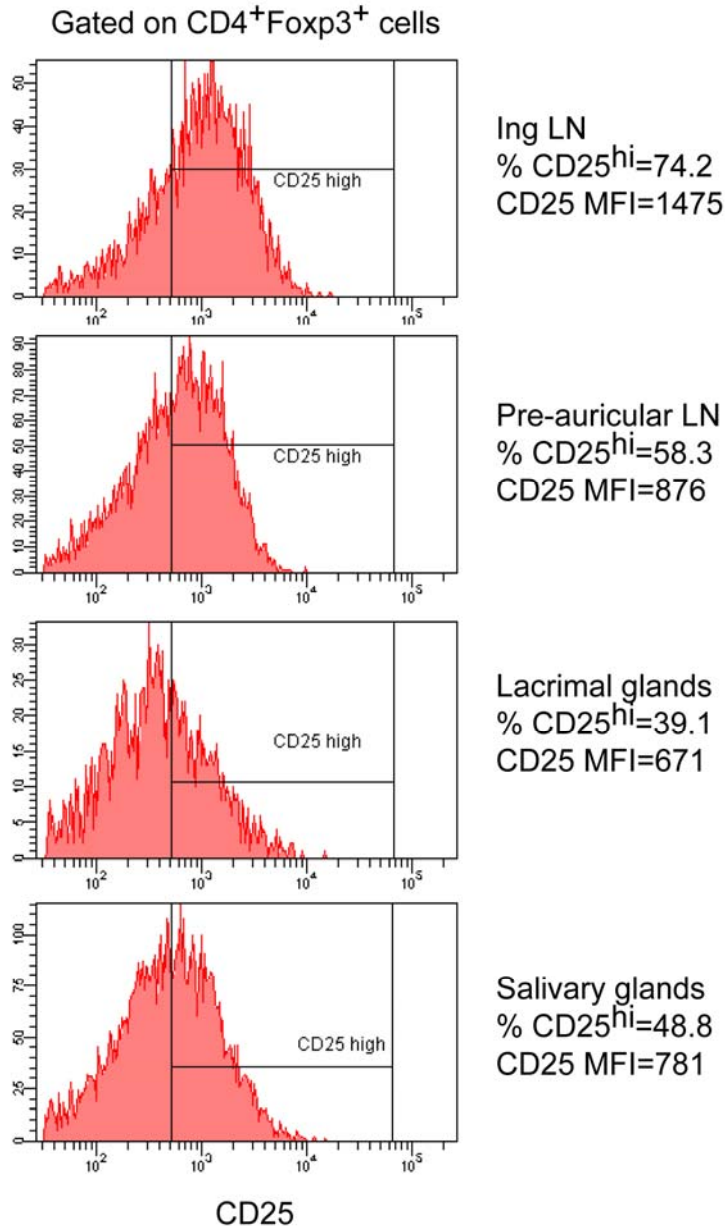
Figure S5. Reduced Expression of CD25 on Infiltrating Tregs in Lachrymal and Salivary Glands of Female NOD Mice

Lachrymal gland and salivary gland infiltrates were extracted by collagenase digestion and enriched by ficoll density gradient. The cells were pooled from four 13 week old female NOD mice and CD25 expression on CD4+Foxp3+ cells were analyzed by flow cytometry. Distal inguinal LNs and lachrymal and salivary gland draining pre-auricular LNs were similarly analyzed for comparison. Percentages of CD25high Tregs and CD25 mean fluorescent intensity (MFI) values are indicated to the right of the histograms for each organ.

Table S1. Summary of Flow Cytometric Comparison of Treg Cell Frequencies in PLNs of 6-Week-Old and New-Onset Diabetic NOD Mice

		6- to 8-week-old			New-onset				
		Mean	SEM	n	Mean	SEM	n	Fold increase	p
PLN	Exp1	7.43	0.5	3	11.35	0.65	2	52.7%	0.01
	Exp2	17.68	0.82	4	26.2	2.78	3	48.2%	0.02
	Exp3	13.63	0.36	3	23.13	3.18	3	69.7%	0.07
ILN	Exp1	6.07	0.40	3	6.45	0.25	2	6.3%	0.48
	Exp2	11.7	0.33	4	11.47	0.42	3	–2%	0.69
	Exp3	9.63	0.18	3	14.53	2.78	3	50%	0.22

Numbers in the Mean columns are percentages of $Foxp3^+$ cells in $CD4^+$ gate.

The anti-Foxp3 antibody used in Exp1 was conjugated to FITC and gave much weaker staining that the APC-labeled anti-Foxp3 used in Exp2 and 3.

p values are calculated using student t test analysis.