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pd-11/pd-12/ mice were backcrossed four generations onto the nod background (n4, n5, or n7). pd-11/pd-12/ mice lacking the nod idd loci (n4, n5, or n7) were fixed for the nod mhc ii allele i-ag7, which was verified by phenotype. wt littermates were used as controls. pd-11/pd-12/ n7 mice on a nod background were fixed for the nod mhc ii allele i-ag7 and verified by phenotype. all mice were aged for diabetes. the mice were backcrossed four generations to nod mice for analyses (n4, n5, or n7) and were fixed for the nod mhc ii allele i-ag7 locus (verified by phenotype). wild-type littermates were used as controls. pd-11/pd-12/ mice lacking the nod idd loci (n4, n5, or n7) were fixed at the nod i-ag7 mhc ii locus. the mice were backcrossed for four generations to nod mice. pd-11/pd-12/ mice lacking the nod idd loci were fixed at the nod mhc ii allele i-ag7 locus. the mice were backcrossed four generations to nod.h2bg7 mice on a nod background. pd-11/pd-12/ mice were fixed for the nod.h2bg7 mhc locus. blood was obtained by tail bleed, and glucose levels were measured with a glucometer (diastix; bayer pharmaceuticals). two pd-1 ligands have been identified in mice, pd-11 and pd-12. in humans, the pd-12 ligand is highly conserved and presents an orthologous gene to the murine pd-12 gene. both pd-1 ligands have a cytoplasmic domain that interacts with a number of proteins, such as shp-2, which inhibits the functions of tcr signaling, and 14-3-3 protein, which is thought to stabilize the inactive form of pd-1, and in this way maintains t cell tolerance. however, the cytoplasmic domain of pd-12 has been shown to interact with and inhibit the transcription factor signal transducer and activator of transcription (stat)6. thus, pd-11 and pd-12 appear to have opposing effects on t cell activation. in mice, pd-11 and pd-12 have distinct expression patterns; pd-11 is expressed in the brain, heart, intestine, kidney, and lung, whereas pd-12 is expressed in the bone marrow, spleen, and thymus. pd-11 is also expressed by activated macrophages and is up-regulated by ifn-gamma treatment. thus, both pd-1 ligands have been shown to be involved in the regulation of t cell activation, and they may play overlapping or distinct roles depending on the tissue microenvironment and cell type.

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the rapid onset of diabetes in pd-l1/pd-l2/ nod mice has important implications for monitoring diabetic patients. pd-l1/pd-l2/ nod mice provide a useful model for the study of human autoimmune disease. the incidence of diabetes can be monitored early in the disease and is completely penetrant in pd-l1/pd-l2/ nod mice, as it is in humans. pd-l1/pd-l2/ mice are also useful for the study of the role of the pd-1/pd-l1 pathway in controlling pathogenic t cell responses. the data presented here demonstrate that pd-l1/pd-l2/ nod mice are defective in the regulation of t cell responses to foreign antigens, resulting in the loss of peripheral tolerance. these pd-l1/pd-l2/ nod mice provide a useful model for the study of defective tolerance to foreign antigens as it occurs in some patients with systemic lupus erythematosus. pd-l1/pd-l2/ mice also provide a useful model for the study of chronic inflammatory diseases such as crohn's disease and rheumatoid arthritis, in which t cell tolerance to self-antigens is impaired. in this study, we demonstrate that pd-l1 and pd-l2 protect against the induction of diabetes in nod mice. these data provide the first genetic evidence that pd-l1 or pd-l2 protect against the development of autoimmune diabetes. this protective function is achieved through two independent mechanisms: pd-l1 protects by inhibiting the function of t cells in the target organ, and pd-l2 controls the activation of apcs that have cross-presented autoantigen. these data are complementary to our previous studies, which showed that pd-l1 but not pd-l2 protects against the development of t1d in nod mice (13) and islet transplant rejection in nod-scid mice (9). pd-l1/pd-l2/ nod mice have a hyperresponsive, hyperproinflammatory, and hyporesponsive phenotype in comparison with wt mice. all of the lagging (hyper)nod mice had a rapid onset of hyperglycemia, similar to wt littermate controls. all lagging (hyper)nod mice are on average hyperglycemic at 12.3 wk of age. pd-l1/pd-l2/ nod female mice were on average diabetic at 8.8 wk of age, whereas pd-l1/pd-l2/ male mice were diabetic at 12.3 wk. the incidence of diabetes was more strongly elevated in female pd-l1/pd-l2/ nod animals than in male pd-l1/pd-l2/ nod animals. these data, in contrast to those from pd-1/ nod mice (23) and nod mice treated with pd-1 or pd-l1blocking antibodies (21), show that pd-l1 and pd-l2 protect against the onset of t1d in nod mice. 5ec8ef588b

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