Interleukin-21 Is Required for the Development of Type 1 Diabetes in NOD Mice

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation
Sutherland, Andrew P.R., Tom Van Belle, Andrea L. Wurster, Akira Suto, Monia Michaud, Dorothy Zhang, Michael J. Grusby, and Matthias von Herrath. 2009. Interleukin-21 Is Required for the Development of Type 1 Diabetes in NOD Mice. Diabetes 58(5): 1144-1155.

Published Version
doi:10.2337/db08-0882

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:4522609

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
OBJECTIVE—Interleukin (IL)-21 is a type 1 cytokine that has been implicated in the pathogenesis of type 1 diabetes via the unique biology of the nonobese diabetic (NOD) mouse strain. The aim of this study was to investigate a causal role for IL-21 in type 1 diabetes.

RESEARCH DESIGN AND METHODS—We generated IL-21R–deficient NOD mice and C57Bl/6 mice expressing IL-21 in pancreatic β-cells, allowing the determination of the role of insufficient and excessive IL-21 signaling in type 1 diabetes.

RESULTS—Deficiency in IL-21R expression renders NOD mice resistant to insulitis, production of insulin autoantibodies, and onset of type 1 diabetes. The lymphoid compartment in IL-21R−/− NOD is normal and does not contain an increased regulatory T-cell fraction or diminished effector cytokine responses. However, we observed a clear defect in autoreactive effector T-cells in IL-21R−/− NOD by transfer experiments. Conversely, overexpression of IL-21 in pancreatic β-cells induced inflammatory cytokine and chemokines, including IL-17A, IL-17F, IFN-γ, monocyte chemoattractant protein (MCP)-1, MCP-2, and interferon-inducible protein-10 in the pancreas. The ensuing leukocytic infiltration in the islets resulted in destruction of β-cells and spontaneous type 1 diabetes in the normally diabetes-resistant C57Bl/6 and NOD × C57Bl/6 backgrounds.

CONCLUSIONS—This work provides demonstration of the essential prodiabeticogenic activities of IL-21 on diverse genetic backgrounds (NOD and C57Bl/6) and indicates that IL-21 blockade could be a promising strategy for interventions in human type 1 diabetes. Diabetes 58:1144–1155, 2009

The nonobese diabetic (NOD) mouse model is the most well-characterized animal model of human type 1 diabetes and has provided important insights into the etiology and pathogenesis of this increasingly prevalent autoimmune disease (1). Rigorous genetic analysis of the NOD background has revealed the existence of multiple defined chromosomal regions known as insulin-dependent diabetes (idd) loci that confer susceptibility to or protection from the development of type 1 diabetes (2). Of the ~15 regions identified, idd3 is of particular importance, because congenic NOD lines containing alleles from protected strains at this locus are significantly less susceptible to diabetes. To date, idd3 is the most potent disease modifying the non–major histocompatibility complex (MHC) locus (3). Therefore, some of the genes within the idd3 interval must play a crucial role in regulating immune destruction of pancreatic β-cells.

Among the several candidate genes within the idd3 locus, interleukin (IL)-21 is of particular interest, because dysregulated IL-21 production and signaling has been found in the NOD mouse (4). IL-21 belongs to the type 1 cytokine family, which includes potent immune modulators such as IL-2, IL-4, IL-7, and IL-15, whose high-affinity receptor complexes all use the common γc receptor subunit (5,6). The specificity of IL-21 signaling is achieved through its specific interaction with the IL-21 receptor subunit, which forms a heterodimer with the γc subunit (7). This receptor complex delivers IL-21 signals to a variety of immune cells including CD4+ and CD8+ T-cells, B-cells, NK cells, NKT cells, and dendritic cells (8–13), all of which can play some role in the pathogenesis of type 1 diabetes in the NOD mouse (14–20). Therefore, the aim of our present study was to better understand the role of IL-21 in type 1 diabetes. We demonstrate that loss of IL-21 signaling, via knockout of the IL-21 receptor, completely abrogates diabetes development on the NOD background. In addition, we demonstrate that overexpression of IL-21 in pancreatic β-cells induces a high incidence of spontaneous type 1 diabetes on the normally diabetes-resistant C57Bl/6 genetic background. Together, these findings clearly underline the potent prodiabeticogenic activity of IL-21.

RESEARCH DESIGN AND METHODS

Mice. All mice were housed in microisolator cages under specific pathogen-free conditions at the Harvard School of Public Health and the La Jolla Institute for Allergy and Immunology. All animal studies were performed according to institutional and National Institutes of Health guidelines for animal use and care. Blood glucose levels were monitored weekly using OneTouch Ultra (LifeScan) or Ascensia Contour glucometers (Bayer). Diabetes in NOD mice was defined as two consecutive blood glucose values ≥250 mg/dl. IL-21 receptor knockout mice were generated by homologous recombination as previously described (11). NOD/Ltj mice were purchased from The Jackson Laboratories, and the IL-21 receptor–null allele was backcrossed to the NOD background for 10 generations. The IL-21 transgenic (IL-21Tg) construct was generated by cloning the full-length murine IL-21 cDNA into a human insulin promoter and 3′ hepatitis B terminator sequence (21). The purified plasmid was linearized using the SacI and HindIII restriction sites, injected into C57Bl/6 fertilized embryos and implanted into pseudopregnant females. Founder lines were identified by Southern blot and maintained as heterozygotes for experimentation.
Tissue isolation, fixation, and immunohistochemical staining. Pancreata were harvested from IL-21R−/− and IL-21R+/+ NOD mice, immersed in OCT Compound (Tissue-Tek, Sakura) and quick-frozen on dry ice. The 6-μm sections were cut at three nonoverlapping levels (200 μm apart) and fixed in acetone for 10 min at room temperature. Sections were incubated for 1 h at room temperature with guinea pig anti-swine insulin (Dako, 1:500), biotin–anti-mouse CD8 (BD clone 53–6.7, 1:50), and biotin–anti-mouse CD4 (BD, clone RM4-5, 1:50). Next, goat anti–guinea pig alkaline phosphatase (Sigma, 1:50) and Avidin-HRP (Vector, 1:2,000) were incubated for 45 min at room temperature. Alkaline phosphatase or horseradish peroxidase (HRP) activity was visualized using Vector Blue Alkaline Phosphatase III (blue signal) and AEC substrate (red precipitates). Slides were mounted without hematoxylin counterstain (Dako Faramount Aqueous Mounting Medium). Islets were scored visually by light microscopy and categorized as no insulitis, peri-insulitis, mild infiltration (<25%), and heavy infiltration and scars.

Pancreata were harvested from IL-21Tg and littermate controls and fixed overnight with 4% paraformaldehyde (Sigma-Aldrich) before routine paraffin embedding. After dewaxing, 6-μm sections were cut at room temperature and treated with 3% H2O2 in MeOH (20 min at room temperature) to quench endogenous peroxidase activity. Antigen retrieval was performed using trypsin or proteinase K digestion. Next, slides were blocked in 1% BSA and 3% normal serum in PBS for 30 min at room temperature. Primary antibodies were incubated overnight at 4°C at the following concentrations: insulin 1:100 (#A0564, Dako), IL-21 (Sigma-Aldrich). Slides were counterstained with hematoxylin before mounting.

RESULTS

Quantitation of serum insulin autoantibodies revealed no differences in CD4+ and CD8+ T-cells were found scattered throughout the islet (Fig. 2C). In contrast, we observed minimal mononuclear cell infiltration in islets of IL-21R+/+ NOD mice up to 40 weeks of age. In keeping with the lack of insulitis, autoimmunity to islet antigens was reduced in IL-21R−/− NOD mice.

Quantitation of serum insulin autoantibodies revealed seropositivity in 10/27 IL-21R−/− NOD mice (8–12 weeks old), in contrast to only 1/20 IL-21R+/+ NOD mice (Fig. 2D). Thus, loss of IL-21 signaling protects NOD mice from diabetes, islet inflammation, and the generation of islet autoantibodies.

We next analyzed the constitution of the lymphoid compartment of various IL-21RNOD genotypes. We found roughly equal splenocyte numbers in IL-21R−/− NOD, IL-21R+/− NOD, and IL-21R−/− NOD at both early (7–9 weeks) and late pre-diabetic stages (12–15 weeks) (data not shown). The proportion of CD4+ and CD8+ T-cells within the lymphocyte population in spleen and the pancreas draining lymph node was not significantly influenced by IL-21R deficiency (Fig. 3A and B; supplementary Fig. 2, found in an online appendix at http://care.diabetescourals/cgi/content/full/db08-0882/DC1). Moreover, the fraction of B-cells and NK cells at 12–15 weeks of age was similar between all genotypes (data not shown).

Enumeration of pancreatic CD4+ and CD8+ T-cells corroborated the insulitis index scores (Fig. 2B) as CD4+ and CD8+ T-cell numbers increased from early to late pre-diabetic stage in IL-21R−/− NOD but were significantly reduced in IL-21R−/− NOD pancreata (Fig. 3A and B, lower panels). We hypothesized that an increased regulatory compartment could explain the observed diabetes resistance of IL-21R−/− NOD mice. Whereas no significant differences in CD4+FoxP3+ Tregs were observed in the spleen of late-stage pre-diabetic mice (Fig. 3C), the Treg...
fraction in the pancreatic lymph nodes of IL-21R−/− NOD mice was reduced (~50%) compared with controls. This may represent a relative reduction of Tregs in IL-21R−/− NOD mice or an increase in IL-21R+/+ NOD related to disease onset (25). Regardless, we conclude that the peripheral lymphoid compartment in IL-21R−/− NOD is essentially normal, with the unexpected exception of reduced Treg numbers in the pancreatic lymph nodes, which suggests that diabetes resistance is not due to an increased regulatory compartment.

Given the absence of obvious cellular defects, we reasoned that modulation of Th effector responses from pathogenic (Th1, Th17) to protective (Th2) may account for diabetes protection in IL-21R−/− NOD mice. Lymphocytes from spleens and pancreatic lymph nodes of 8- to 9-week-old IL-21R+/+ NOD and IL-21R−/− NOD mice were restimulated in vitro with phorbol 12-myristate 13-acetate/ionomycin for 3 h for intracellular cytokine detection by flow cytometry. We found a slight increase in the proportion of CD4+ T-cells that produce IL-17 or interferon (IFN)-γ in the splenic and pancreatic lymph node cells of IL-21R−/− NOD mice (Fig. 4A). We next used enzyme-linked immunosorbent spot assays to confirm these data. Splenocytes from IL-21R+/+ NOD and IL-21R−/− NOD mice were stimulated for 72 h with anti-CD3/anti-CD28 under nonpolarizing conditions. We observed significantly

![Graphs and figures](https://example.com/graphs.png)
increased numbers of IL-17– and IL-4–producing cells in IL-21R−/− NOD splenocytes compared with controls. We also found a trend toward increased IFN-γ– and IL-10– producing cells that failed to reach statistical significance. Thus, whereas there are increases in IL-4 production, concomitant increases in IL-17 and possibly IFN-γ make it unlikely that skewing toward protective Th2 response explains the diabetes resistance in IL-21R−/− NOD mice.

To decipher whether an IL-21R+/+ NOD environment was sufficient to restore the diabetogenic potential of IL-21R−/− NOD lymphocytes, we performed parallel transfers of IL-21R+/− NOD and IL-21R−/− NOD splenocytes into lymphopenic NOD/scid recipients. As previously published, splenocytes from recently diabetic IL-21R+/+ NOD mice induced diabetes upon transfer to NOD/scid mice starting at 3 weeks post-transfer (Fig. 5A). In contrast, transfer of age-matched IL-21R−/− NOD splenocytes could not induce diabetes in NOD/scid mice (Fig. 5A). Immunohistochemistry on pancreatic sections revealed limited islet infiltration by CD4+ and CD8+ T-cells in NOD/scid recipients of IL-21R+/− NOD splenocytes, but abundant infiltration by transferred IL-21R−/− NOD splenocytes. Defective reconstitution of lymphoid space by IL-21R−/− NOD lymphocytes could not explain these observations, as we found equivalent numbers of lymphoid cells in spleen or pancreatic lymph nodes of NOD/scid mice receiving either IL-21R+/+ NOD or IL-21R−/− NOD splenocytes (Fig. 5D). These observations indicate that IL-21R−/− NOD mice lack auto-aggressive splenocytes compared with their wild-type littermates and that lymphopenia-induced proliferation of IL-21R−/− NOD lymphocytes does not confer them with diabetogenic properties.

We showed that pancreatic levels of IL-21 increase during diabetes development in NOD (Fig. 1A) and that loss of IL-21 signaling protects NOD mice from islet infiltration and diabetes development (Fig. 2). We therefore hypothesized that elevated levels of IL-21 would...
exacerbate disease pathogenesis. To test this, we generated transgenic C57Bl/6 mice in which IL-21 is under the control of the human insulin promoter, resulting in pancreatic β-cell–specific overexpression of IL-21 (Fig. 6A).

Next, we measured IL-21 levels by quantitative RT-PCR (Fig. 6B) and by immunohistochemistry using a polyclonal anti-mouse IL-21 antibody (Fig. 6C). These data revealed distinct overexpression of IL-21 mRNA and protein in pancreatic islets of IL-21 transgenic animals.

Analysis of lymphoid compartments revealed splenomegaly and lymphadenopathy in IL-21Tg mice. We identified an ~2.5-fold increase in total cell numbers in spleen (Fig. 6D) and pancreatic draining lymph nodes (Fig. 6E) resultant from expansion of both the T-cell (CD3+) and B-cell (B220+) compartments (data not shown). Most B-cells in our IL-21Tg mice displayed a mature phenotype, while expressing reduced levels of CD21 and CD23 (IgD+, IgM+, CD21lo, CD23lo) (Fig. 6F). Other studies have shown that IL-21 can downregulate surface CD21 and CD23 on B-cells, and expansion of IgD+IgM+CD21loCD23lo B-cells was also observed in other IL-21Tg mouse lines driven by ubiquitous promoters (10). Thus, these data suggest that bioactive IL-21, expressed specifically by pancreatic β-cells, is released systemically from the endocrine pancreas to mediate effects in peripheral lymphoid compartments.

To determine whether IL-21 overexpression resulted in diabetes onset, we monitored blood glucose levels of...
IL-21R–cell–specific overexpression of IL-21 precipitates diabetes in diabetes-resistant C57Bl/6 mice. IL-21Tg mice and wild-type littermate controls were completely protected from diabetes onset (Fig. 7B). To test whether the presence of diabetes susceptibility alleles from NOD influences disease onset, we crossed IL-21Tg mice (C57Bl/6) to NOD mice. We found that IL-21Tg F1 (B6xNOD) mice developed diabetes as early as 3 weeks of age, with a median onset at ~4 weeks and 100% penetrance of disease at 6 weeks (Fig. 7E). This represents a striking acceleration of diabetes onset in IL-21Tg on the mixed B6×NOD versus the B6 background (median onset 4 vs. 22 weeks, respectively; Fig. 7A vs. E). We determined β-cell mass and pancreatic islet infiltration by immunohistochemistry and found a reduced amount of islets and distinct infiltration of the remaining islets between 2 and 3 weeks of age in the IL-21Tg B6×NOD F1 compared with wild-type B6×NOD littermates (Fig. 7F and G). Our data show that one “dose” of NOD-derived alleles exacerbates diabetes in IL-21Tg C57Bl/6 mice.

Next, we used immunohistochemistry to determine which cell subsets infiltrate the islets in IL-21Tg C57Bl/6 mice. We analyzed the presence of B-cells (B220−), CD4+ cells (CD4+), NK cells (LGL-1+), macrophages (F4/80+), and dendritic cells (CD11c−) in islet infiltrates from pre-diabetic (8–10 weeks; Fig. 8A, top panel) and diabetic IL-21Tg cohorts (24 weeks; Fig. 8A, bottom panel). We observed more severe infiltration by all cell types in IL-21Tg versus littermate controls, and in diabetic versus pre-diabetic mice (Fig. 8B), corroborating our data in Fig. 7. The infiltrates in the pre-diabetic IL-21Tg cohort predominantly contained F4/80+ macrophages but also CD4+ and dendritic cells. In diabetic IL-21Tg mice, the infiltrates consisted mostly of macrophages and contained focal accumulation of CD4+ cells, B-cells, dendritic cells, and NK cells. We reasoned that the distinct pattern of infiltration could result in part from the production of cytokines and chemokines. Therefore, we performed quantitative RT-PCR on pancreatic tissue from IL-21Tg and littermate controls, which revealed significantly increased production of IFN-γ, IL-17A, and IL-17F in the pancreas of IL-21Tg mice (Fig. 8C). In addition, we found a significant increase in monocyte chemoattractant protein (MCP)-1, MCP-2, and IFN-inducible protein (IP)-10 production (Fig. 8D). Thus, pancreatic β-cell–specific overexpression of IL-21 results in the production of inflammatory cytokines and chemokines and predominant infiltration of the islets by macrophages and CD4+ T-cells.

FIG. 4. IL-21R−/− NOD mice display altered cytokine production profiles. A: IFN-γ and IL-17 production by CD4+ T-cells upon 3 h phorbol 12-myristate 13-acetate/ionomycin stimulation of freshly isolated IL-21R+/+ and IL-21R−/− NOD splenocytes. B: enzyme-linked immunosorbent spot analysis of IFN-γ, IL-17, IL-4, and IL-10 production upon 72 h of in vitro unpolarized anti-CD3/28 stimulation of freshly isolated IL-21R+/+ and IL-21R−/− NOD splenocytes.

DISCUSSION

In this study, we demonstrate a causal relationship between IL-21 production and type 1 diabetes. First, IL-21 production increases as spontaneous diabetes develops in the NOD model. Second, IL-21R–deficient NOD mice are protected from type 1 diabetes. Third, β-cell–specific overexpression of IL-21 precipitates diabetes in diabetes-resistant C57Bl/6 mice.

Type 1 diabetes pathogenesis in the NOD model consists of a sequence of stages. Initially, islet antigens are released during postnatal remodeling of the pancreas and captured by migratory and resident antigen-presenting cell that prime anti-islet T-cells in the pancreatic draining lymph nodes (20,26–28). At an early stage, macrophages are
recruited to the islets (29) and are a necessary cellular component of diabetes pathogenesis (30). Next, chemotactic factors, produced by β-cells in response to inflammatory stimuli, attract mononuclear cells to the islets, particularly CD4+ and CD8+ effector T-cells. The transition from nondestructive islet inflammation to a β-cell-destructive state is a key event that precipitates type 1 diabetes (18,31). Because IL-21R is broadly expressed throughout the immune system and other nonhematopoietic lineages (6,32–35), there are multiple time points and sites of action for IL-21 during the pathogenesis of type 1 diabetes.

We show here that IL-21 levels are increased in the pancreas as NOD mice develop diabetes and that CD4+ and CD8+ T-cells infiltrating the pancreas can respond to local IL-21 as they express IL-21R. Our data are consistent with recent studies by the labs of Leonard and Sarvetnick (36,37) showing that IL-21R−/− NOD mice are protected from insulitis and type 1 diabetes. Similar to Spolski et al. (37), we find unaltered numbers of T-cells, B-cells, and NK cells in IL-21R−/− NOD lymphoid organs (Fig. 3 and data not shown). In contrast to ours and other studies, Datta and Sarvetnick detected higher lymphocyte numbers in IL-21R−/− NOD mice, interpreting this as a normalization of IL-21–induced, type 1 diabetes–promoting lymphopenia (4,36). We see no differences in the expansion of IL-21R+/+ NOD and IL-21R−/− NOD splenocytes when transferred to lymphopenic NOD/scid recipients (Fig. 5D), yet IL-21R−/− NOD splenocytes still fail to induce diabetes. We therefore think it unlikely that IL-21 catalyzes diabetes development by regulating homeostatic proliferation.

Given that T-cell numbers and responses are intact in IL-21R−/− mice (8,11), we hypothesized that altered cytokine production may partially account for the protection from type 1 diabetes. Our analyses show that production of various effector cytokines was not impaired in IL-21R−/− NOD mice (Fig. 4A and B). One of these cytokines, IL-17, has recently been shown to be a necessary cellular component of diabetes pathogenesis in NOD (38), and recent studies identify IL-21 as an amplifying factor for Th17 responses (39,40). Spolski et al. (37) identify defective polarization toward the Th17 lineage in IL-21R−/− NOD lymphocytes and reason that defective IL-17 production may explain diabetes resistance in IL-21R−/− NOD mice. We (data not shown) and others (39) find similarly defective in vitro Th17 polarization using IL-21R−/− T-cells. Moreover, our data show increased IL-17 production in the pancreas of β-cell–specific IL-21 overexpressing mice (Fig. 8D). However, we show increased numbers of IL-17–producing cells in IL-21R−/− NOD mice when cells are restimulated directly ex vivo, which is likely to be more reflective of the in vivo context. Thus, we conclude that reduced IL-17 production in IL-21R−/− NOD mice is unlikely to be the mechanism for the protection from type 1 diabetes.

The reduced frequency of insulin autoantibodies and insulitis in IL-21R−/− NOD mice (Fig. 2B and D) shows
that the anti-islet response is impaired at multiple levels. Reduced autoantibody levels may reflect impairments in CD4+ T helper cell function or antibody production in the absence of IL-21R (9, 41, 42). Anti-islet IL-21R−/− T-cells may be primed ineffectively or possess inherent defects in migration to islet tissue. Since IL-21R−/− NOD mice have normal or fewer numbers of regulatory T-cells (Fig. 3C), and the function of these cells is not altered (37), it is unlikely that increased regulatory function explains the reductions in autoimmunity. Transfer experiments using diabetogenic T-cell receptor–transgenic T-cells may elucidate the existence of defects in priming or trafficking and are the subject of ongoing studies.

Although IL-21R deficiency protects diabetes-prone NOD mice from type 1 diabetes, β-cell–specific overexpression of IL-21 causes severe diabetes in otherwise diabetes-resistant C57Bl/6 mice. Few other models of cytokine overexpression in pancreatic islets cause diabetes of similar severity (43, 44). The phenotype of IL-21Tg mice most closely resembles that of IFN-γ Tg mice in terms of onset and severity of disease. The IFN-γ Tg model is both T-cell and macrophage dependent (21, 45, 46). Similar to the IFN-γ Tg model, the high numbers of macrophages in the islet infiltrates of IL-21Tg mice suggest an important role for macrophages, since macrophage-derived inflammatory cytokines and reactive oxygen species are directly

---

**FIG. 6.** Expression of IL-21 in pancreatic islets through an insulin promoter transgenic construct leads to increased cellularity of lymphoid organs and altered expression of B-cell maturation markers in IL-21Tg mice. A: Murine IL-21 was cloned into the transgenic construct under the control of the human insulin promoter and HBS (hepatitis B virus) terminator sequences. B: IL-21 mRNA levels were measured in pancreatic tissue from IL-21Tg and littermate controls by quantitative RT-PCR. C: Immunohistochemical staining for paraffin embedded pancreatic tissue with an IL-21–specific polyclonal antibody (left panel Tg−, right panel Tg+, a representative islet is marked with an arrow). Original magnification: ×20. Total cell numbers in spleen (D) and pancreatic draining lymph nodes (E) (*P < 0.05) and IgD vs. IgM staining (F) of B220+ cells (top panels) and frequencies of mature (IgM− IgD+) and marginal zone/transitional (IgM+ IgD+) B-cells are shown. CD23 vs. CD21 staining of IgM+ cells (bottom panels) and mean fluorescence intensity for each marker is shown on the relevant axis (n = 4 for all experiments shown). (A high-quality digital representation of this figure is available in the online issue.)
toxic to β-cells (47). In vitro stimulation of macrophages with IL-21 enhances their T-cell priming capacity (48); thus, phagocytosis of damaged islets and presentation of β-cell antigens to CD4+ T-cells may cause enhanced killing of islets in the IL-21Tg model. In IL-21Tg pancreatic tissue, we showed upregulation of chemokine transcripts such as MCP-1, MCP-2, and IP-10, which recruit inflammatory cells such as macrophages and CXCR3+ T-cells (Fig. 8E) (49). Previous studies identified β-cells as an important source of chemokines during diabetes pathogenesis, but our experiments have failed to identify IL-21R expression on β-cells (supplementary Fig. 1). Regardless, we believe that
IL-21–dependent inflammatory chemokine production could be an important element of the pathogenesis of type 1 diabetes and partially explain the protection afforded by IL-21R deficiency in the NOD model (33,50,51).

In conclusion, we demonstrate a critical role of IL-21 for diabetes pathogenesis in animal models. The disease-promoting activities of IL-21 involve the recruitment of CD4+ cells and macrophages to inflamed islets and may...
reflect events that occur in response to IL-21 production by infiltrating cells. In addition, the partial protection from diabetes in IL-21R−/− NOD mice shows the sensitivity of the diabeticogenic response to alterations in IL-21 signaling and, by inference, IL-21 levels. Thus, both of our experimental models suggest that the use of IL-21 blocking agents, antibodies, or IL-21R-Fc fusion proteins has potential therapeutic value for the prevention or treatment of human type 1 diabetes.

ACKNOWLEDGMENTS
A.P.R.S. is a CJ Martin Fellow of the National Health and Medical Research Council of Australia. T.V.B. is a fellow of the Belgian American Educational Foundation and funded by the Brehm Center for Type 1 Diabetes Research and Analysis.

No potential conflicts of interest relevant to this article were reported.

We thank Kirsten Sigrist, Jennifer Donovan, Diana Pascual, Therese Junti, Jeanette Liao, and the staff at the Harvard School of Public Health Animal Testing Facility for assistance with animal care.

REFERENCES
1. Anderson MS, Bluestone JA. The NOD mouse: a model of immune dysregulation. Annu Rev Immunol 2005;23:447–485
2. Wicker LS, Todd JA, Peterson LB. Genetic control of autoimmune diabetes in the NOD mouse. Annu Rev Immunol 1995;13:179–200
3. Wicker LS, Todd JA, Prins JB, Podolni PL, Renjilian RG, Peterson LB. Resistance alleles at two non-major histocompatibility complex-linked insulin-dependent diabetes loci on chromosome 3, Idd3 and Idd10, protect nonobese diabetic mice from diabetes. J Exp Med 1994;180:1705–1713
4. King C, Ilie A, Koelsch K, Sarvetnick N. Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. Cell 2004;117:265–277
5. Mehta DS, Wurster AL, Grusby MJ. Biology of IL-21 and the IL-21 receptor. Immunol Rev 2004;202:84–95
6. Leonard WJ, Spolski R. Interleukin-21: a modulator of lymphoid proliferation, apoptosis and differentiation. Nat Rev Immunol 2005;5:688–698
7. Parrish-Novak J, Dillon SR, Nelson A, Hammond A, Sprecher C, Gross JA, Johnson J, Maddy K, Xu W, West J, Schrader S, Birkhead S, Heipel M, Brandt C, Kuiper JL, Kramer J, Conklin D, Pressnell SR, Berry J, Shiota F, Bort S, Hambly K, Murdi S, Clegg C, Moore M, Grant FJ, Lotton-Day C, Gilbert T, Rayond F, Ching A, Yao L, Smith D, Webster P, Whitmore T, Maurer M, Kaushansky L, Holly RD, Foster D. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. Nature 2004;431:57–63
8. Wurster AL, Rodgers VL, Satoskar AR, Witters MJ, Young DA, Collins M. IL-21-mediated “active tolerance” in the nonobese diabetic mouse shows the sensitivity of the diabeticogenic response to alterations in IL-21 signaling and, by inference, IL-21 levels. Thus, both of our experimental models suggest that the use of IL-21 blocking agents, antibodies, or IL-21R-Fc fusion proteins has potential therapeutic value for the prevention or treatment of human type 1 diabetes.

IL-21 IN NOD MICE
13. Brandt K, Duflo-Paus S, Foster DC, Ruckert R. Interleukin-21 inhibits dendritic cell activation and maturation. Blood 2003;102:4996–4998
14. Christiansson SW, Shultz LD, Leiter EH. Adaptive transfer of diabetes into immunodeficient NOD.scid/scid mice: relative contributions of CD4+ and CD8+ T-cells from diabetic versus prediabetic NOD.NON-Thy-1a donors. Diabetes 1993;42:44–55
15. Herbelin A, Gombert JM, Lepault F, Bach JP, Chatenoud L. Mature mainstem TCR alpha beta+CD4+ thymocytes expressing L-selectin mediate “active tolerance” in the nonobese diabetic mouse. J Immunol 1998;161:2020–2028
16. Serreze DV, Leiter EH, Christianson GJ, Greiner D, Roopendaal DC. Major histocompatibility complex class I-deficient NOD-R2mmun mice are diabetic and insulitis resistant. Diabetes 1994;43:505–509
17. Serreze DV, Fleming SA, Chapman HD, Richard SD, Leiter EH, Tisch RM. Lymphocytes are critical antigen-presenting cells for the initiation of T-cell-mediated autoimmune diabetes in nonobese diabetic mice. J Immunol 1998;161:3912–3918
18. Poirier L, Benoist C, Mathis D. Natural killer cells distinguish innocuous and destructive forms of pancreatic islet autoimmunity. Proc Natl Acad Sci U S A 2004;101:8102–8107
19. Cain JA, Smith JA, On-dr Jk, Wang B, Katz JD. NKT cells and IFN-gamma establish the regulatory environment for the control of diabeticogenic T cells in the nonobese diabetic mouse. J Immunol 2006;176:1645–1654
20. Turley S, Poirier L, Hattori M, Benoist C, Mathis D. Physiological beta cell death triggers priming of self-reactive T cells by dendritic cells in a type-1 diabetes model. J Exp Med 2003;198:1527–1537
21. Sarvetnick N, Liggitt D, Pitts SL, Hansen SE, Stewart TA. Insulin-dependent diabetes mellitus induced in transgenic mice by ectopic expression of class II MHC and interferon-gamma. Cell 1988;52:773–782
22. Seewald S, Thomas HE, Egnraa M, Christen U, Wofle T, Rodrigo E, Coon B, Michelena B, Cay TW, von Herrath MG. Virus-induced autoimmune diabetes: most beta-cells die through inflammatory cytokines and not perform from autoreactive (anti-viral) cytotoxic T-lymphocytes. Diabetes 2000;49:1801–1809
23. Yu L, Robles DR, Abiru N, Kaur P, Revers M, Kelemen K, Eisenbarth GS. Early expression of antiinsulin autoantibodies of humans and the NOD mouse: evidence for early determination of subsequent diabetes. Proc Natl Acad Sci U S A 2006;103:1701–1706
24. Jin H, Carrio R, Yu A, Malek TR. Distinct activation signals determine whether IL-21 generates beta cell somitulation, growth arrest, or Bin-dependent apoptosis. J Immunol 2004;173:657–665
25. Tang Q, Adams JY, Penaranda C, Melli K, Piaggio E, Sgueroidus E, Piciriello CA, Salomon BL. Bhaestone JA. Central role of defective interleukin-2 production in the triggering of islet autoimmune destruction. Immunity 2006;28:687–697
26. Trudeau JD, Dutz JP, Arany E, Hill DJ, Fields WE, Finegood DR. Neonatal beta-cell apoptosis: a trigger for autoimmune diabetes? Diabetologia 2000;43:1–7
27. Hohlund P, Mintem J, Watzlting C, Heath W, Benoist C, Mathis D. Initiation of autoimmune diabetes by developmentally regulated presentation of islet cell antigens in the pancreatic lymph nodes. J Exp Med 2000;191:1391–1399
28. Zhang Y, O’Brien B, Trudeau J, Tan R, Santamaria P, Dutz JP. In situ beta cell death promotes priming of diabeticogenic CD8+ T lymphocytes. J Immunol 2002;168:1466–1472
29. Yoon JW, Jun HS, Santamaria P. Cellular and molecular mechanisms for the initiation and progression of beta cell destruction resulting from the collaboration between macrophages and T cells. Autoimmunity 1998:27:109–122
30. Jun HS, Yoon CS, Zbytnuk L, van Rooijen N, Yoon JW. The role of macrophages in T-cell mediated autoimmune diabetes in nonobese diabetic mice. J Exp Med 1999;189:347–358
31. Andre-Schmutz I, Hiddelang C, Benoist C, Mathis D. Cellular and molecular changes accompanying the progression from insulin to diabetes. Eur J Immunol 1999;29:245–255
32. Caruso R, Fina D, Peluso I, Fantini MN, Tosti C, Del Vecchio Blanco G, Paoluzi OA, Caprili F, Andrei F, Stolfi C, Romano M, Ricci V, Macdonald TT, Pallone F, Monteleone G. IL-21 is highly produced in Helicobacter pylori-infected gastric mucosa and promotes gelatinases synthesis. J Immunol 2002;168:5907–5915
33. Caruso R, Fina D, Peluso I, Stolfi C, Fantini MC, Gioia V, Caprili F, Del Vecchio Blanco G, Paoluzi OA, Maclonald TT, Pallone F, Monteleone G. A functional role for interleukin-21 in promoting the synthesis of the T-cell chemoattractant, MIP-3alpha, by gut epithelial cells. Gastroenterology 2007;132:166–175
34. Monteleone G, Caruso R, Fina D, Peluso I, Gioia V, Stolfi C, Fantini MC, Caprili F, Tersigili R, Alessandrini L, Macdonald TT, Pallone F. Control of matrix metalloproteinase production in human intestinal fibroblast by interleukin-21. Gut 2000;46:1774–1780
35. Leonard WJ, Zeng R, Spolski R. Interleukin-21: a cytokine/cytokine receptor system that has come of age. J Leukoc Biol 2008;8:82–92
36. Datta S, Sarvetnick NE. IL-21 limits peripheral lymphocyte numbers through T cell homeostatic mechanisms. PLoS ONE 2008;3:e3118
37. Spolski R, Kashyap M, Robinson C, Yu Z, Leonard WJ. IL-21 signaling is critical for the development of type I diabetes in the NOD mouse. Proc Natl Acad Sci USA 2008;105:14028–14033
38. Jain R, Tartar DM, Gregg RD, Divekar RD, Bell JJ, Lee HH, Yu P, Ellis JS, Hoeman CM, Franklin CL, Zaghouani H. Innocuous IFNγ induced by adjuvant-free antigen restores normoglycemia in NOD mice through inhibition of IL-17 production. J Exp Med 2008;205:207–218
39. Korn T, Bettelli E, Gao W, Awasthi A, Jager A, Strom TB, Oukka M, Kuchroo VK. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. Nature 2007;448:484–487
40. Bettelli E, Korn T, Kuchroo VK. Th17: the third member of the effector T cell trilogy. Curr Opin Immunol 2007;19:652–657
41. Vogelzang A, McGuire HM, Yu D, Sprent J, Mackay CR, King CVogelzang A et al. A fundamental role for interleukin-21 in the generation of T follicular helper cells. Immunity 2008;29:127–137
42. Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L, Wang YH, Watowich SS, Jetten AM, Tian Q, Dong C. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity 2008;29:138–149
43. Rabinovitch A. An update on cytokines in the pathogenesis of insulin-dependent diabetes mellitus. Diabetes Metab Rev 1998;14:129–151
44. Rabinovitch A, Suarez-Pinzon WL. Cytokines and their roles in pancreatic islet beta-cell destruction and insulin-dependent diabetes mellitus. Biochem Pharmacol 1998;55:1130–1149
45. Sarvetnick N, Shizuru J, Liggett D, Martin L, McIntyre B, Gregory A, Parslow T, Stewart T. Loss of pancreatic islet tolerance induced by beta-cell expression of interferon-gamma. Nature 1990;346:844–847
46. Gu D, O’Reilly L, Molony L, Cooke A, Sarvetnick N. The role of infiltrating macrophages in islet destruction and regrowth in a transgenic model. J Autoimmun 1995;8:483–492
47. Dahlen E, Dawe K, Ohiason L, Hedlund G. Dendritic cells and macrophages are the first and major producers of TNF-alpha in pancreatic islets in the nonobese diabetic mouse. J Immunol 1998;160:3585–3593
48. Ruckert R, Bullone-Paus S, Brandt K. Interleukin-21 stimulates antigen uptake, protease activity, survival and induction of CD4+ T cell proliferation by murine macrophages. Clin Exp Immunol 2008;151:487–495
49. Yamada S, Oikawa Y, Sakai G, Atsumi Y, Maruyama T, Shinada A. Expression levels of CXC chemokine receptors 3 are associated with clinical phenotype of type 1 diabetes. Ann N Y Acad Sci 2006;1079:186–189
50. Martin AP, Rankin S, Pitchford S, Charo IF, Furtado GC, Lira SA. Increased expression of CCL2 in insulin-producing cells of transgenic mice promotes mobilization of myeloid cells from the bone marrow, marked insulitis, and diabetes. Diabetes 2008;57:3025–3033
51. Christen U, Von Herrath MG. IP-10 and type 1 diabetes: a question of time and location. Autoimmunity 2004;37:273–282