Interleukin-4 but not Interleukin-10 Protects Against Spontaneous and Recurrent Type 1 Diabetes by Activated CD1d-Restricted Invariant Natural Killer T-Cells

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In nonobese diabetic (NOD) mice, a deficiency in the number and function of invariant natural killer T-cells (iNKT cells) contributes to the onset of type 1 diabetes. The activation of CD1d-restricted iNKT cells by α-galactosylceramide (α-GalCer) corrects these deficiencies and protects against spontaneous and recurrent type 1 diabetes. Although interleukin (IL)-4 and IL-10 have been implicated in α-GalCer–induced protection from type 1 diabetes, a precise role for these cytokines in iNKT cell regulation of susceptibility to type 1 diabetes has not been identified. Here we use NOD.IL-4−/− and NOD.IL-10−/− knockout mice to further evaluate the roles of IL-4 and IL-10 in α-GalCer–induced protection from type 1 diabetes. We found that IL-4 but not IL-10 expression mediates protection against spontaneous type 1 diabetes, recurrent type 1 diabetes, and prolonged syngeneic islet graft function. Increased transforming growth factor-β gene expression in pancreatic lymph nodes may be involved in α-GalCer–mediated protection in NOD.IL-10−/− knockout mice. Unlike the requirement of IL-7 and IL-15 to maintain iNKT cell homeostasis, IL-4 and IL-10 are not required for α-GalCer–induced iNKT cell expansion and/or survival. Our data identify an important role for IL-4 in the protection against type 1 diabetes by activated iNKT cells, and these findings have important implications for cytokine-based therapy of type 1 diabetes and islet transplantation. Diabetes 53:1303–1310, 2004

Autoimmune type 1 diabetes spontaneously develops in nonobese diabetic (NOD) mice and is characterized by T-cell infiltration (insulitis) followed by a progressive destruction of pancreatic islet β-cells. Current evidence suggests that immune dysregulation leading to defective regulatory T-cell function elicits type 1 diabetes (1,2). In NOD mice, effectors CD4+ T-cells of type 1 diabetes are Th1 cells, and regulatory CD4+ T-cells may include Th2 cells (2–4). The deficiency in interleukin (IL)-4 production by T-cells mediates immune dysregulation and was proposed to be a causal factor of type 1 diabetes in NOD mice (2,5). Murine IL-4 restores normal NOD T-cell proliferative responsiveness in vitro (1,2,5), and anti-CD28 and IL-4 treatments protect NOD mice from type 1 diabetes (1,2,6). Despite this and other evidence for a role of IL-4 secreting Th2 cells in protection against type 1 diabetes (7–9), a “protective” role for Th2 cells has not been demonstrated (3). Other regulatory T-cell subsets, including CD4+CD25+ T-cells and invariant natural killer T-cells (iNKT cells) also mediate protection from type 1 diabetes (10–14).

iNKT cells are identified by the coexpression of an invariant T-cell receptor (TCR) and various natural killer (NK) cell-related surface markers, including NK1.1, high levels of cytokine (IL-4, interferon [IFN]-γ) production upon activation, and the recognition of glycolipid molecules bound to the nonclassic major histocompatibility complex (MHC) class I molecule CD1d (13,14). TCR-α chains expressed by iNKT cells consist of Va14-Jo18 gene segments and exhibit a strong bias for Vβ8.2 followed by Vβ2 and Vβ7 in mice. Although the natural ligands recognized by iNKT cells remain unknown, the synthetic glycolipid α-galactosylceramide (α-GalCer) isolated from Agelas mauritanius marine sponges can activate iNKT cells. Upon activation with α-GalCer, iNKT cells can regulate susceptibility to autoimmune disease and tumor surveillance (13,14).

We and others (13–18) have shown that deficiencies in iNKT cell number and function mediate the development of type 1 diabetes in NOD mice and that α-GalCer–induced iNKT cell activation corrects these deficiencies and reduces the incidence of spontaneous and recurrent type 1 diabetes in these mice. The latter protection from type 1 diabetes is associated with a polarized Th2 milieu marked
by elevated IL-4 and IL-10 and reduced IFN-γ levels in the spleen and pancreas as well as increased IL-10R transcription in the spleen (15,16). Neutralization of IL-10 activity by an anti–IL-10R mAb treatment in vivo abrogates the protective effect of α-GalCer in cyclophosphamide (CY)-induced type 1 diabetes (15). Administration of anti–IL-4 and anti–IL-10 mAbs blocks protection from type 1 diabetes afforded by adoptive transfer of TCR-CD4−CD8− thymocytes containing iNKT cells (19). While these observations suggest that protection from type 1 diabetes by α-GalCer is mediated by IL-10 and/or IL-4, the mechanism(s) of iNKT cell regulation of susceptibility to type 1 diabetes remains unknown (14).

Here we use NOD.IL-4−/− and NOD.IL-10−/− mice to show that IL-4 but not IL-10 mediates protection against spontaneous type 1 diabetes, recurrent type 1 diabetes, and prolonged syngeneic islet graft function by α-GalCer–activated iNKT cells. Thus, we have identified an important role for IL-4 in the protection against spontaneous and recurrent type 1 diabetes by α-GalCer–activated CD1d-restricted iNKT cells.

RESEARCH DESIGN AND METHODS

NOD/Scid and NOD/Scid mice were bred in a specific pathogen-free barrier facility at The Robarts Research Institute (London, ON, Canada). Insulitis and type 1 diabetes are detectable by 4–6 weeks and 4–6 months of age, respectively, in our colony of female NOD mice. The incidence of type 1 diabetes in this colony is 25–30% at 15 weeks of age and >80% by 25 weeks. NOD.IL-4−/− and NOD.IL-10−/− mice were obtained from The Jackson Laboratory (Bar Harbor, ME), and their knockout phenotypes were confirmed by PCR typing (20).

Assessment of diabetes. Mice were monitored for type 1 diabetes by measurement of blood glucose levels (BGLs) twice weekly using a Glucometer (Bayer, Toronto, ON). NOD mice that displayed BGL >11.1 mmol/l on two consecutive readings were indicative of the onset of type 1 diabetes.

CY-induced diabetes. Pre-diabetic (8–9 weeks old) female NOD and NOD.IL-10−/− mice were injected intraperitoneally with CY (200 mg/kg) (Sigma, St. Louis, MO) on day 0 and day 10. Diabetes onset was monitored every other day for 30 days postinjection.

Adoptive cell transfer. NOD and NOD.IL10−/− mice (12–13 weeks old) were treated with α-GalCer or vehicle on days 0, 2, 4, 6, and 8. Spleens were collected on day 10, and spleen single-cell suspensions were prepared. Spleonocytes (1 × 10⁶ cells) pooled from each group (n = 5 mice) were transferred intraperitoneally to 5- to 6-week-old NOD/Scid recipient mice (15).

Detection of iNKT cells by flow cytometry. NOD mice (9–10 weeks old) were treated with α-GalCer or vehicle (kindly supplied by Kirin Brewery Co., Gunma, Japan) on days 0, 2, 4, 6, and 8, as described (15). Tissues were collected on day 10, and spleen and pancreatic lymph node (PLN) single-cell suspensions were prepared. Spleonocytes (1 × 10⁶ cells) pooled from each group (n = 5 mice) were injected intraperitoneally to 6-week-old NOD/Scid recipient mice (15).

Flow cytometry analyses were conducted using a FACSCalibur and CELLQuest software (BD Biosciences, San Jose, CA). The specificity of staining in all tissues was verified by examining tissues from NOD.CD1d−/− mice.

Treatment of mice with α-GalCer. For protection against spontaneous type 1 diabetes, 10-week-old NOD, NOD.IL-4−/−, and NOD.IL-10−/− mice were treated with α-GalCer (5 µg/dose) every other day for five doses and then boosted at 13–14 weeks of age. For protection against CY-induced diabetes in NOD and NOD.IL-10−/− mice, α-GalCer (5 µg/dose) or vehicle was injected intraperitoneally on days 0, 2, 4, 6, and 8 after CY challenge. For islet transplantation, islet graft recipients were treated with α-GalCer (5 µg/dose) on days −1, 2, 7, 14, and 21, as reported (15).

Islet transplantation. Pancreatic islets were isolated from 3- to 4-week-old male NOD mice by collagenase digestion and discontinuous density gradient purification. After overnight culture, islets (n = 400) were transplanted to the renal subcapsular space of newly (<7 days) diabetic (spontaneous or CY-induced) female NOD mice (15).

RESULTS

α-GalCer protection against spontaneous type 1 diabetes is mediated by IL-4. Modulation of iNKT cells by α-GalCer reduces the incidence of spontaneous type 1 diabetes in NOD mice even when administered after the onset of invasive insulitis (15,16). We therefore investigated whether α-GalCer treatment initiated at 10 weeks of age protects against spontaneous type 1 diabetes in NOD.IL-4−/− and NOD.IL-10−/− mice. Consistent with our previous report (15), α-GalCer induced protection against type 1 diabetes in NOD mice compared with vehicle-treated mice (P < 0.05), as the incidence of spontaneous type 1 diabetes was reduced from 80 to 42% at 32 weeks of age (Fig. 1A). However, the incidence of type 1 diabetes in α-GalCer–treated NOD.IL-4−/− mice was not significantly reduced compared with vehicle-treated NOD.IL-4−/− mice (85 vs. 65%, P > 0.05). α-GalCer–treated NOD.IL-10−/− mice did not develop type 1 diabetes during 15–25 weeks of age, when colitis is evident in these mice (unpublished observations). These data suggest that prevention of spontaneous type 1 diabetes by α-GalCer is mediated by IL-4 and not IL-10.

![Graph](image-url)
**FIG. 2.** Protection against CY-induced diabetes by α-GalCer is not mediated by IL-10. Female NOD (A), NOD.IL-10-/- (B), and NOD.CD1d-/- (C) mice (8–9 weeks old, n = 8–15) were challenged with CY (200 mg/kg) at day 0 and day 10 and received α-GalCer (●) or vehicle (○) (5 μg·mouse⁻¹·dose⁻¹) intraperitoneally at days 0, 2, 4, 6, and 8 after CY challenge. Mice were screened for glycemia every other day starting at day 15, and mice with BGls of >11.1 mmol/l on two consecutive readings were considered diabetic. Data from one of three representative and reproducible experiments are shown.

α-GalCer protects against CY-induced type 1 diabetes in NOD.IL-10-/- mice. Previously, we reported that α-GalCer-mediated protection against CY-induced type 1 diabetes is associated with the ability of splenocytes from CY-challenged and α-GalCer–treated female NOD mice to secrete more IL-4 and IL-10 and less IFN-γ than splenocytes from vehicle-treated mice upon in vitro restimulation with α-GalCer (15). To further evaluate the significance of increased IL-10 levels induced upon iNKT cell activation, as NOD.IL-10-/- mice spontaneously develop colitis starting at 12–15 weeks of age, we investigated whether NOD mice deficient in IL-10 expression are protected from CY-induced type 1 diabetes upon treatment with α-GalCer. Consistent with our previous study, Fig. 2A shows that α-GalCer treatment protects from CY-induced type 1 diabetes in NOD mice, with the incidence of type 1 diabetes being 35% in α-GalCer–treated NOD mice and 75% in vehicle-treated NOD mice (P < 0.01), respectively. NOD.IL-10-/- mice were also protected against CY-induced type 1 diabetes (P < 0.01) (Fig. 2B), and the level of protection exceeded that detected in NOD wild-type (Fig. 2A) mice (P < 0.01). α-GalCer treatment did not protect from type 1 diabetes in NOD.CD1d-/- mice, which are deficient in iNKT cells (Fig. 2C). An adoptive transfer model of type 1 diabetes was also used to test the role of IL-10 in the development of type 1 diabetes upon iNKT cell activation. Similar to our previous report that spleen cells from α-GalCer–treated NOD mice have a diminished capacity to transfer type 1 diabetes to NOD. Scid mice (15), spleen cells from NOD.IL-10-/- mice harvested 2 weeks after α-GalCer treatment also demonstrated a reduced capacity to transfer type 1 diabetes (Fig. 3A and B). Thus, in contrast to the requirement for IL-4 in GalCer-mediated protection against type 1 diabetes, IL-10 does not appear to be required for this protection.

**FIG. 3.** Spleen cells from α-GalCer–treated NOD and NOD.IL-10-/- mice do not transfer type 1 diabetes. Splenocytes (10⁶ cells) from individual α-GalCer–treated (●, n = 8) and vehicle–treated (○, n = 8) NOD (A) and NOD.IL-10-/- (B) mice (11–12 weeks old) were collected 2 weeks after the last injection of α-GalCer and transferred into NOD. Scid recipients (n = 8–10). Mice were screened for glycemia twice weekly starting at 5 weeks post transfer. Data are from one of two representative and reproducible experiments.

α-GalCer–induced prolongation of syngeneic islet graft depends on the activity of IL-4 but not IL-10. Prolongation of pancreaticoduodenal graft survival in non-recurrent spontaneous diabetic BB rats is associated with the proliferation of donor-derived iNKT cells in hepatic and splenic tissues and higher serum levels of IL-4 (22). The acceptance of xenogeneic islet grafts in mice is also dependent on iNKT cells (23). iNKT cells therefore appear to enhance transplanted islet syngeneic graft and xenograft survival.

Previously, we showed that α-GalCer treatment prolongs graft function significantly in newly diagnosed diabetic NOD recipients of syngeneic islet transplants (15). Since a recurrent autoimmune response in islet graft recipients may be prevented by treatment with IL-4 and IL-10 (24), we determined whether IL-4 and/or IL-10 mediates the ability of α-GalCer–activated iNKT cells to prolong syngeneic islet graft survival and prevent recurrent type 1 diabetes. While islet grafts failed in 90% of control vehicle-treated NOD spontaneous diabetic recipients by
day 8 posttransplantation, grafts in 90% of α-GalCer-treated NOD mice were still functional at this time, as evidenced by the maintenance of euglycemia (Fig. 4A). In contrast, treatment of newly diagnosed NOD.IL-4−/− diabetic mice with α-GalCer did not prolong graft function in islet transplant recipients compared with vehicle-treated NOD.IL-4−/− diabetic mice (Fig. 4B). Moreover, whereas 100% of islet grafts failed by day 12 posttransplantation in all vehicle-treated NOD and NOD.IL-4−/− mice and α-GalCer–treated NOD.IL-4−/− mice, islet grafts survived as long as 25 days in ~40% of α-GalCer–treated NOD mice (Fig. 4A). However, the lack of IL-4 expression does not accelerate graft rejection, and syngeneic islet grafts survive in vehicle-treated NOD.IL-4−/− mice for a period similar to that in vehicle-treated NOD mice. Since NOD.IL-10−/− mice spontaneously develop colitis starting at 12–15 weeks of age, we used CY-induced diabetic mice as islet graft recipients. All islet grafts in α-GalCer–treated CY-induced NOD.IL-10−/− diabetic mice retained function at 20–30 days postransplant, but these grafts failed within 10 days postransplant in all vehicle-treated NOD.IL-10−/− mice (Fig. 4C). Some α-GalCer–treated NOD.IL-10−/− mice with functional islet grafts had to be sacrificed due to the development of colitis. Removal of grafts in NOD and NOD.IL-10−/− mice with long surviving grafts resulted in a prompt return to a hyperglycemic state (Fig. 5A and B), which indicated that the maintenance of euglycemia in α-GalCer–treated diabetic recipient mice is islet graft dependent. Thus, α-GalCer–prolonged syngeneic islet graft function occurs in an IL-4–dependent and IL-10–independent manner.

α-GalCer–treated NOD.IL-4−/− and NOD.IL-10−/− mice differ in their cytokine gene expression profile compared with wild-type NOD mice. To further determine how IL-4 and not IL-10 mediates α-GalCer–mediated protection from type 1 diabetes, we conducted cDNA array analyses of the gene expression of inflammatory cytokines in the PLNs of NOD.IL-4−/− and NOD.IL-10−/− mice. Compared with wild-type NOD mice, vehicle-treated NOD.IL-4−/− mice show an increased expression of IL-10RB, CXCR5, CCR6, CCR7, TARC, BLC, MIG, and SDF-1 and decreased expression of CXCR4 (Table 2). In contrast, vehicle-treated NOD.IL-10−/− mice show an increased expression of IL-2, CXCR5, CCR7, Scy6, BLC, MIG, SDF-1, and tumor necrosis factor (TNF)-R1 and a decreased expression of IL-11 and CXCR4 compared with NOD mice. Interestingly, transforming growth factor (TGF)-β gene expression was found to be elevated in vehicle-treated NOD.IL-10−/− mice compared with NOD and NOD.IL-4−/− mice. In general, the gene expression profiles in α-GalCer–

FIG. 5. Maintenance of euglycemia in α-GalCer–treated diabetic recipients. Removal of grafts in α-GalCer–treated NOD (A) and NOD.IL-10−/− (B) mice with long surviving grafts resulted in a prompt return to a hyperglycemic state. Unilateral nephrectomy to remove the grafts from mice treated with α-GalCer (see Fig. 2A and C) is denoted by arrows.

FIG. 4. Prolongation of syngeneic islet graft function by α-GalCer treatment requires IL-4 but not IL-10 expression. A and B: Prolongation of islet graft survival conferred by α-GalCer is dependent on IL-4 expression. Newly diagnosed spontaneous diabetic female NOD (A, n = 10) and NOD.IL-4−/− (B, n = 5–6) mice were transplanted under the kidney capsule with ~400 syngeneic islets from 3- to 4-week-old male NOD donors. Graft recipients were treated with 5 μg/dose of vehicle or α-GalCer on days −1, 2, 7, 14, and 21. Recipients were monitored every other day for their BGLs. C: Prolongation of islet graft induced by α-GalCer treatment is not dependent on IL-10 expression. Newly diagnosed CY-induced diabetic female NOD.IL-10−/− mice were transplanted with islets, treated with α-GalCer (n = 6) or vehicle (n = 4), and monitored for their BGLs as described in A and B above.
development of type 1 diabetes (28,29), our data suggest and that SDF-1/CXCR4 interaction and LT-β may be associated with deficient IL-4 expression and contribute to the requirement of IL-4 in α-GalCer-mediated protection from type 1 diabetes.

**IL-4 and IL-10 are not required for the expansion or survival of iNKT cells.** To determine whether IL-4 or IL-10 is required for α-GalCer to promote the expansion and/or survival of iNKT cells in secondary lymphoid organs, we examined the frequency of iNKT cells in the spleen and PLNs of α-GalCer–treated and −untreated NOD.IL-4−/− and NOD.IL-10−/− mice. iNKT cells were tracked by double-staining with an anti-TCRβ mAb and CD1d tetramers loaded with α-GalCer (15). The frequencies of iNKT cells in the spleen and PLNs of wild-type NOD and all three knockout NOD mouse strains were increased significantly after 8 days of α-GalCer treatment compared with the frequencies observed in vehicle-treated mice (P < 0.01). However, no significant differences were observed in the frequencies of iNKT cells in the spleens of the α-GalCer–treated wild-type and cytokine knockout NOD mice (Table 1, experiment 1). Whereas α-GalCer stimulated about a twofold increase in the frequency of iNKT cells in the PLN of NOD.IL-4−/− mice relative to that in NOD.IL-10−/− and wild-type NOD mice, the frequencies observed in NOD and NOD.IL-10−/− mice were similar (P > 0.05). No significant differences were detected in the spleen and PLNs of vehicle-treated mice (Table 1, experiment 1). Similar frequencies of iNKT cells were also found in the spleen and PLNs of NOD and NOD.IL-10−/− mice at 30 days after CY and α-GalCer treatment (Table 1, experiment 2). Thus, unlike the requirement of IL-7 and IL-15 to
deficient IL-10 expression and thus decrease the incidence of type 1 diabetes. On the other hand, decreased expression of IL-11 and increased expression of SDF-1/CXCR4 and LT-β may be associated with deficient IL-4 expression and contribute to the requirement of IL-4 in α-GalCer-mediated protection from type 1 diabetes.

### Table 1

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<th>Strain</th>
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<th>PLN</th>
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<td>NOD</td>
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<td>NOD.IL-10−/−</td>
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<tr>
<td>NOD</td>
<td>1.3 ± 0.3</td>
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Data are means ± SD. *Female NOD, NOD.IL-4−/−, and NOD.IL-10−/− mice (9–10 weeks old, n = 5–7) were treated with vehicle or α-GalCer (5 μg/dose) on days 0, 2, 4, 6, and 8. On day 10, spleen and PLNs were collected, and iNKT cells were stained with α-GalCer–loaded CD1d tetramers and anti–TCRβ. †P < 0.01 vs. NOD and NOD.IL-10−/− mice. ‡Female NOD and NOD.IL-10−/− mice (8–9 weeks old) were challenged with CY (200 mg/kg) on day 0 and day 10 and received α-GalCer or vehicle (5 μg·mouse−1·dose−1 i.p.) on days 0, 2, 4, 6, and 8 after CY challenge. On day 30, spleen and PLNs were collected from nontreated mice and double-stained with an anti–TCRβ antibody and CD1d tetramers loaded with α-GalCer.

treated NOD.IL-4−/− and NOD.IL-10−/− mice were very similar to those from vehicle-treated control mice, with the exception that IL-11 gene expression was decreased in NOD.IL-4−/− mice (Table 2). NOD.IL-10−/− mice displayed increased IL-6R, MDC, MCP-2, and TGFβ expression when compared with NOD mice (Table 2). Given that IL-11 and TGFβ elicit protection against type 1 diabetes (25–27) and that SDF-1/CXCR4 interaction and LT-β mediate the development of type 1 diabetes (28,29), our data suggest that the α-GalCer–mediated protection seen in NOD.IL-10−/− mice may be due to the increased expression of TGFβ. Elevated TGFβ expression may compensate for

### Table 2

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Total RNA was extracted from the PLNs of α-GalCer– or vehicle-treated mice and was used to analyze gene expression by GEArray. A greater than twofold change in both two separate experiments was considered significant. The average expression value was shown. NS, less than a twofold change.

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maintain iNKT cell homeostasis (30), IL-4 and IL-10 do not appear to be required for α-GalCer–induced iNKT cell expansion and/or survival.

**DISCUSSION**

This study provides direct evidence for the requirement of IL-4 but not IL-10 in the protection against spontaneous type 1 diabetes and prolongation of syngeneic islet graft function by α-GalCer–activated iNKT cells. In the case of IL-10, this observation is consistent with the preliminary results of a recent report that IL-10 may not be necessary for iNKT-mediated protection against type 1 diabetes induced by transfer of diabeticogenic BDC2.5 T-cells to Vα14Cα/– NOD mice (31).

Curiously, a deficiency of Vα14+/iNKT cells in CD1d-deficient NOD mice is causally linked to the induction of type 1 diabetes, and yet this Vα14+/iNKT cell deficiency does not alter the Th1 and Th2 cytokine profiles in these mice (14). Moreover, an accelerated accumulation of CCR4+ diabeticogenic T-cells in pancreatic islets is associated with Vα14+/iNKT cell dysfunction, indicating that Vα14+/iNKT cells may have another important role in the prevention of autoaggressive T-cell recruitment to sites of inflammation (32). It is noteworthy that mature myeloid CD8α+ dendritic cells (DCs) accumulate in the PLNs only after treatment with α-GalCer, and that this accumulation protects against type 1 diabetes. Transfer of these myeloid DCs into NOD mice significantly protects them against type 1 diabetes (17). Therefore, an immunoregulatory role for Vα14+/iNKT cells in the recruitment of tolerogenic myeloid DCs (CD8α+ myeloid DC) to PLN, rather than a Th1/Th2 cytokine imbalance, is suggested (14,17).

Unexpectedly, our findings appear to differ from our previous report that neutralization of IL-10 activity by an anti–IL-10R antibody in vivo abrogates the protective effect of α-GalCer in CY-induced type 1 diabetes (15). One explanation for this difference may be that the timing of anti–IL-10R administration or production of IL-10 may differ during the development of type 1 diabetes, as reflected by an altered biological activity of IL-10 in vivo. Another explanation is that IL-10 can exhibit seemingly paradoxical effects on susceptibility to type 1 diabetes in NOD mice. Systemic treatment of young NOD mice with IL-10 protects against type 1 diabetes (33,34). In contrast, anti–IL-10 mAb treatment of young NOD mice reduces the severity of insulitis (35). Whereas BALB/c mice that express an IL-10 transgene in their islet β-cells do not develop type 1 diabetes, NOD/IL-10 mice that express an IL-10 transgene in islet β-cells develop type 1 diabetes at an accelerated rate (36,37). IL-10 contributes early to the pathology of type 1 diabetes via a CD8+ T-cell–dependent pathway, as anti-CD8 antibody–mediated depletion of CD8+ T-cells inhibits the onset of type 1 diabetes without attenuating the severity of insulitis (38). In addition, IL-10 can also block the regulatory activity in vitro of a CD3+ CD4+CD8+ T-cell subset (39), amplify CD4+ T-cell production of the islet β-cell destructive IFN-γ and TNF-α cytokines (40), exacerbate graft rejection (41–43), and mediate the development of certain autoimmune lymphoproliferative disorders (37,44,45). These sets of findings raise the possibilities that IL-10 may inhibit and/or delete activated regulatory T-cells, such as iNKT cells, and that a lack of IL-10 expression in NOD.IL-10–/– mice may enhance the level of protection from type 1 diabetes induced by iNKT cell activation. Further experimentation is required to test these possibilities.

In addition, our cDNA array data indicate that the increased level of TGF-β gene expression in the PLNs of α-GalCer–treated NOD.IL-10–/– mice compared with α-GalCer– or vehicle-treated NOD and NOD.IL-4–/– mice may be responsible for the protection observed in these mice. Consistent with our findings in IL-10–deficient NOD mice, Sturian et al. (46) observed increased plasma levels of TGF-β in IL-10–deficient mice when studying colitis. Recently, a role for TGF-β in NKT cell function was described, as Gansuvd et al. (47) demonstrated that rhesus NKT cells can secrete large amounts of TGF-β and are in a semianergic state that leads to polarization toward a Th3 regulatory cell phenotype with regulatory/suppressive function in vitro. It is interesting that we observed a greater increase in TGF-β gene expression in IL-10–deficient NOD mice relative to NOD mice after α-GalCer–induced iNKT activation, which suggests that activated iNKT cells might overexpress TGF-β in the absence of IL-10 expression. This may be explained by the observation that IL-10 and TGF-β tend to be cosecreted at sites of inflammation. A study by Kitani et al. (48) using intranasal administration of a TGF-β–encoding plasmid showed that while TGF-β rapidly induces IL-10 production, IL-10 does not induce the secretion of TGF-β, suggesting that these two cytokines might be coordinately regulated. Thus, in α-GalCer–treated NOD.IL-10–/– mice, an increase in TGF-β expression in the PLNs may either compensate for the absence of expression of IL-10 or may be the result of a lack of negative regulation induced by the cytokine. In contrast, in α-GalCer–treated NOD.IL-4–/– mice, a diminished level of IL-11 expression is detectable and no compensatory effect of TGF-β in the PLNs is apparent. This may explain why α-GalCer–treated NOD.IL-10–/– mice are not protected from type 1 diabetes and further supports the notion that α-GalCer–mediated protection is IL-4 dependent.

A recent report demonstrated that CXCR5/BLC and CCR7/MDC interactions are crucial factors in the development of autoimmunity (49,50). We found that expression of CXCR5, BLC, CCR7, and MDC are elevated in vehicle-treated NOD.IL-4–/– and NOD.IL-10–/– mice compared with vehicle-treated NOD mice. Nonetheless, we did not observe any accelerated onset of spontaneous type 1 diabetes in these mice. On the other hand, complete protection was obtained in α-GalCer–treated NOD.IL-10–/– mice. Thus, it is possible that increased CXCR5/BLC and CCR7/MDC interactions do not mediate the lack of protection from type 1 diabetes in α-GalCer–treated NOD.IL-4–/– mice.

Our observations also differ from the result that α-GalCer is unable to protect B6.IL-4–/– and B6.IL-10–/– mice against experimental autoimmune encephalomyelitis (51). Therefore, the mechanism of iNKT cell–dependent protection from different autoimmune diseases may depend on the genetic background in which a given disease develops.

In new onset diabetic NOD mice, the grafting of syngeneic islets in the renal subcapsular area restores euglycemia. However, due to a recurrent autoimmune response, the graft is rejected within 10 days of transplantation and
hyperglycemia returns. Renal subcapsular islet grafts in NOD mice are infiltrated predominantly by Th1 cells, and various types of immunomodulation (e.g., treatment with IL-4, CFA, or insulin) elicit a Th1- to Th2-type shift and suppress recurrent type 1 diabetes (2). Here we show that α-GalCer–prolonged syngeneic islet graft function occurs in an IL-4–dependent and IL-10–independent manner, similar to the ability of IL-4 but not IL-10 to mediate α-GalCer–induced protection against spontaneous type 1 diabetes. Thus, due to these differential cytokine requirements, activated iNKT cells may operate via different mechanisms to restore tolerance during the development of spontaneous and recurrent type 1 diabetes.

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