Blockade of Tumor Necrosis Factor–Related Apoptosis-Inducing Ligand Exacerbates Type 1 Diabetes in NOD Mice

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Type 1 Diabetes in NOD Mice

The tumor necrosis factor (TNF) superfamily of receptors and ligands play an important role in immune surveillance and tolerance. Recently, a relatively new member of the TNF superfamily, TNF-related apoptosis-inducing ligand (TRAIL), was identified and shown to have anti-tumor activity. TRAIL shows structural and functional similarities to CD95L, including the use of FADD as an adaptor molecule. TRAIL is a type 2 membrane protein, and the unique feature of TRAIL with respect to CD95L and TNF-α is considered to be its ability to induce apoptosis of most tumor cells without displaying toxic effects on normal cells and tissues in vitro (1).

Preclinical studies in mice and nonhuman primates have shown that administration of a recombinant soluble form of TRAIL suppresses the growth of TRAIL-sensitive tumor xenografts with no apparent systemic toxicity (2,3). Unlike FasL or TNF-α, which have one or two receptors, TRAIL interacts with five different receptors, demonstrating the complexity of this novel ligand. In humans, TRAIL can bind two death-inducing receptors, TRAIL-R1 [death receptor four (DR4)] and TRAIL-R2 [death receptor five (DR5)], which have functional death domains that result in apoptosis through a FADD/caspase-dependent pathway (4).

TRAIL is also recognized by three other receptors, the decoy receptors TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2) as well as the soluble receptor osteoprotegerin (TRAIL-R5), which contain nonfunctional death domains. The functions of these receptors are unknown, but they may act by sequestering ligands in a competitive manner and thereby diminish apoptotic signals (5,6). In mice, only one receptor, DR5, has been described as sharing some structural homology with human TRAIL-R2 (7). TRAIL and its receptors are constitutively expressed in a variety of cell types, including T-cells, B-cells, natural killer (NK) T-cells, NK cells, and dendritic cells (8–12).

Although little is known about the nonapoptotic events induced by TRAIL, recent studies have elucidated some physiological roles of TRAIL in vivo. TRAIL gene-targeted mice are more susceptible to experimental and spontaneous tumor metastasis (13,14), implicating TRAIL in the control of tumor growth in vivo. In addition, we have previously reported that the blocking of endogenous TRAIL activity with soluble DR5 (sDR5) accelerates the onset of both experimental autoimmune encephalomyelitis (EAE) and autoimmune arthritis (15,16). Thus, one of the functions of TRAIL in vivo may be to maintain immune homeostasis and downregulate immune responses that lead to autoimmune disease (16). The maintenance of organ and tissue homeostasis and immune tolerance are controlled by several mechanisms, including cell proliferation and apoptosis (17,18).

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AICD, activation-induced cell death; CFSE, 5- and 6-carboxy-fluorescein diacetate succinimidyl ester; cdk, cyclin-dependent kinase; CY, cyclophosphamide; Dr5, diabetogenic T-cells; EAE, experimental autoimmune encephalomyelitis; HSA, human serum albumin; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; NK, natural killer; NIA, National Institute on Aging; STZ, streptozotocin; TCR, T-cell receptor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; TUNEL, transferase-mediated dUTP nick-end labeling.
In this study, we tested the hypothesis that TRAIL may be involved in the development of type 1 diabetes in nonobese diabetic (NOD) mice, which possess an immunopathological profile similar to that of the human disease. We found that TRAIL gene expression is upregulated in pancreatic islets during the development of type 1 diabetes and in TNF-α + interferon (IFN)-γ-treated Min6 islet β-cells. However, TRAIL cannot induce the apoptosis of freshly isolated pancreatic islets or Min6 cells. Using sDR5, we found that chronic endogenous TRAIL blockage can exacerbate the development of type 1 diabetes. We also demonstrated that TRAIL inhibits the proliferation of diabeticogenic T-cells by suppressing interleukin (IL)-2 production and increasing p27kip1 expression. This inhibition can be rescued in vitro by exposure to exogenously added IL-2. These findings indicate that TRAIL is a potent immune regulator of type 1 diabetes, and that this immunoregulation may include the induction of anergy of diabeticogenic T-cells.

**RESEARCH DESIGN AND METHODS**

**Mice.** NOD and NOD.Scid mice were bred in a specific pathogen-free barrier facility at The John P. Robarts Research Institute (London, ON, Canada).

**Cyclophosphamide-induced diabetes.** Prediabetic (7- to 8-week-old) female NOD mice were challenged intraperitoneally with sDR5 (150 μg/ml) or human serum albumin (HSA) (Sigma-Aldrich) every other day for 6 days before intraperitoneal injection of cyclophosphamide (CT) (200 mg/kg) (Sigmaca) and 8 days after CT injection. Mice also received a second dose of CT at day 10. Diabetes onset was monitored three times weekly starting at day 8 after CT injection until day 35.

**Production of soluble TRAIL receptor.** sDR5 was produced using the Pichia pastoris expression system, as reported (15). Purified sDR5 was found to contain 1–2 ng of lipopolysaccharide (LPS) per mg of protein, as determined by a Limulus amebocyte lysate assay. This was comparable with control HSA, which contains 1–4 ng LPS per mg of protein.

**Preparation of islet cells and MTT assay.** Pancreata of 3-week-old NOD male mice were treated with collagenase P (Boehringer) for 6 min at 37°C with vigorous shaking in PBS supplemented with 5% FCS. Islets were handpicked under an inverted microscope and then treated with trypsin/EDTA (Invitrogen-Gibco) for 20 min at 37°C to obtain single-cell suspensions. Islet cells or Min6 cells were first cultured in flat-bottom 96-well plates at 4 × 10^4 cells/well in Dulbecco’s modified Eagle’s medium with 15% fetal bovine serum (Invitrogen-Gibco). TNF-α (Alexis) was added 48 h later and incubated for an additional 24 h. IFN-γ (20–100 units/ml) (BD PharMingen; each at 20 ng/ml) was added to the culture to induce apoptosis. MTT (0.5 mg/ml) was added during the last 3–4 h of culture to monitor cell viability. The resulting formazan crystals were solubilized in 0.04 N HCl (100 μl) in isopropanol. The optical density was read at 595 and 655 nm using a microplate reader (Bio-Rad). The reduction in optical density induced by TRAIL with or without TNF-α + IFN-γ treatment was used as a measure of viability, which was normalized to cells incubated in medium only that were considered to be 100% viable.

**Splenic T-cell isolation and proliferation assay.** Splenic T-cells were isolated on T-cell enrichment columns (R & D Systems) to a purity of ≥95% as detected by flow cytometric analysis of CD3 surface expression. Cells (5 × 10^6 per well) were cultured (six replicates) in complete RPMI-1640 medium (Invitrogen-Gibco) supplemented with 0, 25, 50, or 100 ng/ml of soluble TRAIL (Alexis) for 48 h in 96-well round-bottom plates. Plates were coated with an anti-CD3 mAb (1.5 or 10 μg/ml; Cedarlane). In another experiment, IL-2 (10 ng/ml) was added after 48 h and cultured for an additional 48 h. [H]-Thymidine (1 μCi/well; Amersham) was added 18 h before the cells were harvested (Tomtec cell harvester; Fisher Scientific), and proliferation was quantified by liquid scintillation (1450 Microbeta; Fisher Scientific).

**T-cell adoptive transfer.** Female NOD,Scid mice (n = 5–6/group) at 7–9 weeks of age were injected intraperitoneally with diabeticogenic spleen T-cells (5 × 10^6) from newly diabetic NOD female mice treated with TRAIL, as described (19). Alternatively, NOD mice were sacrificed at 35 days after CY plus sDR5 or HSA treatment, and splenocytes were tested for IL-2 production and cell proliferation by different concentrations of the GAD65 autoantigen.

**CFSE staining.** Diabeticogenic splenic T-cells were resuspended (20–30 × 10^6 cells/ml), incubated (10 min, 37°C) with 10 μmol/5- and 6-carboxy-fluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes), washed with PBS, resuspended in complete medium in a 6-well plate, and incubated for 48 h in 5% CO2 at 37°C. After harvesting the cells, they were washed in PBS and fixed in 500 μl of 2% paraformaldehyde. Cell division was analyzed using a FACScan and CellQuest software (BD Biosciences).

**Transferase-mediated dUTP nick-end labeling assay.** Transferase-mediated dUTP nick-end labeling (TUNEL) assay was performed to determine the percentage of cells undergoing apoptosis after treatment with TRAIL using a kit (Roche) as per the manufacturer’s suggestion. Diabeticogenic T-cells and islet cells were cultured for 24 h with either TRAIL at different concentrations or with TNF-α + IFN-γ (each at 20 ng/ml) in controls.

**GE cDNA array analysis.** Diabetogenic spleen T-cells were cultured in the presence or absence of soluble TRAIL (50 ng/ml) for 18 h, and Min6 islet β-cells were cultured in the presence or absence of TNF-α + IFN-γ (each at 20 ng/ml) for 24 h. Total RNA was extracted using a RNeasy protect mini kit (Qiagen). Aliquots of RNA (5 μg) were used to analyze gene expression using a mouse cell cycle or apoptosis GEArray cDNA array (SuperArray), as described (19).

**National Institute on Aging immunomicroarray analysis.** Pancreatic islet RNA was isolated using a RNeasy protect mini kit (Qiagen) from NOD mice at 8–13–15 weeks of age or within 1 week after the onset of type 1 diabetes. In control samples, islet RNA was isolated from 13-week-old NOD,Scid mice. Gene expression profiling of pancreatic islets was analyzed during the development of type 1 diabetes by the National Institute of Aging (NIA) (NIH, Bethesda, MD) immunomicroarray, as described (20).

**Immunoblotting.** At the indicated conditions and times of culture, cell lysates prepared in a protease inhibitor cocktail (Boehringer Mannheim) were prepared and equal amounts of protein (25 μg/sample) analyzed by 10% SDS-PAGE, transferred onto nitrocellulose membranes, and immunoblotted with an anti-p27kip1 mAb (BD Bioscience).

**Statistical analysis.** The Student’s t test was used to analyze the spleen T-cell proliferation, IL-2 production, and TUNEL and MTT assays. For analyses of cumulative diabetes frequencies, a Wilcoxon test was used. P values <0.05 were considered significant.

**RESULTS**

TRAIL is overexpressed in pancreatic islets during diabetes development in NOD mice. In NOD mice, peri-insulitis begins at 3–4 weeks of age and is followed by a nondestructive insulitis at 8–10 weeks of age. An invasive and destructive insulitis then appears at 3–4 months, which is followed by the onset of overt type 1 diabetes by 5–6 months of age (18). To address whether TRAIL is involved in the development of type 1 diabetes in NOD mice, we first examined the gene expression of TRAIL in pancreatic islets at various stages during progression to type 1 diabetes. Total islet RNA was isolated from female NOD mice at 8–9 (insulitis) and 13–15 (conversion from nondestructive to destructive insulitis) weeks of age and from newly diagnosed diabetic NOD mice (≥20 weeks old). RNA from NOD,Scid islets at 13 weeks of age served as a control. In Fig. 1A, cDNA microarray analyses show that TRAIL gene expression in pancreatic islets at 15 weeks of age and at onset of type 1 diabetes is upregulated four- to sixfold compared with that observed in 8- to 9-week-old NOD and 13-week-old NOD,Scid mice. Thus, the expression of TRAIL in islets is upregulated at the stage of destructive insulitis during the development of type 1 diabetes.

Since pancreatic islets contain different cell types, including inflammatory cells in which TRAIL may be upregulated during the development of type 1 diabetes, apoptotic islet β-cells may not be a source of TRAIL. We tested this possibility by analyzing the apoptosis gene expression
profile in TNF-α + IFN-γ-treated Min6 cells using a GE cDNA apoptosis array. Consistent with our data obtained using the NIA cDNA array, the gene expression of TRAIL was found to be increased about threefold in treated compared with untreated Min6 cells (Fig 1B). These findings further suggest that TRAIL expression is upregulated in TNF-α + IFN-γ-induced apoptotic islet β-cells, and that islet β-cells may be a source of TRAIL in pancreatic islets with destructive insulitis. Alternatively, the expression of cytokines induced by islet inflammation may also lead to TRAIL expression before and/or independently of apoptosis.

**TRAIL does not induce apoptosis in NOD islet cells.** Apoptosis is the main route of islet β-cell death that leads to type 1 diabetes in NOD mice, and is associated with the increased expression of FasL and the pro-inflammatory type 1 cytokines IL-1β, TNF-α, and IFN-γ (21–23). Exposure of human, rat, or mouse purified β-cells to IL-1β in vitro, in combination with IFN-γ and/or TNF-α, induces severe functional suppression and death by apoptosis (23). Based on the report that TRAIL can induce apoptosis in human hepatocytes and brain cells (24,25) and that human pancreas also expresses TRAIL and related receptors (26), we reasoned that enhanced TRAIL gene expression in pancreatic islets may contribute to the apoptosis of islets during the onset of type 1 diabetes. Thus, we examined whether TRAIL induces the apoptosis of NOD pancreatic islets. NOD islets were isolated from 3-week-old male NOD mice and then treated with different concentrations of TRAIL. Unexpectedly, TRAIL did not induce the apoptosis of pancreatic islet cells, in contrast to the apoptosis of islets noted upon exposure to TNF-α + IFN-γ (Fig. 2A). Similarly, TRAIL did not induce the apoptosis of Min6 cells compared with that obtained upon TNF-α + IFN-γ treatment (Fig. 2A and B). These data are consistent with the notion that different pancreatic cell lines vary in their sensitivity to TRAIL, even though TRAIL can induce the apoptosis of most tumor cells (26).

Our findings indicate that, unlike Fas/FasL and TNF-α, TRAIL cannot induce islet β-cell apoptosis and the upregulation of TRAIL in islets may not contribute to islet cell apoptosis.

**TRAIL blockade exacerbates the onset of type 1 diabetes.** TRAIL can suppress the activity of inflammatory cells involved in the pathogenesis of EAE and autoimmune arthritis (15,16). To investigate whether TRAIL blockade in vivo influences effector T-cell function during the development of type 1 diabetes, we used an adoptive transfer model to determine whether TRAIL blockade inhibits the transfer of type 1 diabetes to NOD.Scid recipients. Diabetic spleen T-cells from newly diag-
increased incidence of CY-induced type 1 diabetes in NOD mice treated with sDR5 (Fig. 3B). About 80% of NOD mice developed type 1 diabetes at day 11 in sDR5-treated mice after CY injection compared with the onset of type 1 diabetes at day 19 in HSA-treated mice. The incidence of type 1 diabetes reached 100 and 80%, respectively, in mice treated with sDR5 or HSA. Similar results were also obtained in streptozotocin (STZ)-induced diabetes in TRAIL-deficient mice on the C57BL/6 genetic background. Severe insulitis and massive islet destruction were observed in TRAIL-deficient mice (27). Thus, TRAIL blockade exacerbates the onset of type 1 diabetes, suggesting that TRAIL may play a crucial role in the prevention of diabetes onset possibly by suppression of the activity of diabetogenic T-cells.

**TRAIL suppresses diabetogenic T-cell proliferation by inhibition of cell cycle progression.** While TNF-α and FasL mediate lymphocyte activation-induced cell death (AICD) (28), TRAIL was generally thought to be incapable of inducing AICD in lymphocytes (15,28). However, recent studies suggest that subsets of T-cells are sensitive to TRAIL-induced AICD (11,12). The latter result raises the possibility that the effect of sDR5 on type 1 diabetes might be explained by its ability to block TRAIL-induced apoptosis of diabetogenic T-cells.

To test this possibility, we assessed the capacity of TRAIL to induce AICD of NOD diabetogenic spleen T-cells after adoptive transfer into NOD.Scid recipients by examining the death and survival of these T-cells in the presence of differing concentrations of soluble TRAIL (sTRAIL) in vitro. Results from an MTT assay of cell viability (Fig. 2C) and TUNEL assay of apoptosis (Q.-S.M., C.M., T.L.D., unpublished data) showed that TRAIL does not induce the AICD of diabetogenic T-cells. However, significant decreases (P < 0.01) in plate-bound anti-CD3 (1.5 or 10 μg/ml)-induced in vitro proliferative responses of diabetogenic T-cells were observed in the presence of sTRAIL at concentrations of 50 and 100 ng/ml (Fig. 3C). These findings are consistent with those of others (29) and our previous report (15,16) that TRAIL does not induce apoptosis in autoimmune T-cells but rather inhibits their activation. Interestingly, in response to TRAIL, whereas the proliferative capacity of spleen T-cells from 4-week-old NOD mice was not altered upon activation by a weak T-cell receptor (TCR) signal (anti-CD3, 1.5 μg/ml), a strong TCR signal (anti-CD3, 10 μg/ml) resulted in the inhibition of proliferation (Fig. 3C). These data suggest that 1) TRAIL-mediated protection from type 1 diabetes may be related to the hypoproliferation rather than enhanced AICD of diabetogenic T-cells, and 2) diabetogenic T-cells are more sensitive to TRAIL-induced inhibition of proliferation.

To further investigate the effect of TRAIL on T-cell proliferation, we also quantitated the proliferative capacity of CFSE-labeled diabetogenic spleen T-cells stimulated in the presence of sTRAIL. CFSE is an intracellular fluorescent dye that has been used to effectively assay cellular division. With each cell division, the level of CFSE fluorescence is reduced to half, which can be measured and quantified by flow cytometry (30). CD3/CD28 costimulation of NOD diabetogenic spleen T-cells for 48 h in the absence or presence of sTRAIL (50 ng/ml) showed that
sTRAIL blocked the division of the majority of the T-cells (Fig. 4A). This resulted in a threefold decrease in the percentage of proliferating T-cells treated with sTRAIL. In contrast, in the absence of sTRAIL 60% of the T-cells underwent two rounds of division (Fig. 4B). These findings further demonstrate that sTRAIL inhibits the progression and activation of NOD diabetic spleen T-cells.

**TRAIL increases the gene and protein expression of cyclin-dependent kinase inhibitor p27kip1.** Previously, we demonstrated that TRAIL can arrest inflammatory cells at G1 phase of the cell cycle (15) but did not investigate the mechanism involved. To drive cell cycle progression, cyclins associate with the activated isoform of a specific cyclin-dependent kinase (cdk) to form an active holoenzyme, which is regulated by a specific cdk inhibitor that associates with a cyclin/cdk complex (31). The major inhibitors of cyclin/cdk complexes are members of the INK (p15INK4b, p16INK4a, p18INK4c, and p19INK4 days) and the cip/kip (p21cip1, p27kip1 and p57kip2) families (31).

To determine the intracellular signaling pathways associated with the TRAIL-mediated inhibition of cell cycle progression of NOD diabetic T-cells, cell cycle gene array and Western blotting analyses were conducted. Total RNA from NOD diabetic T-cells exposed or not to sTRAIL was used to generate cDNA probes that were hybridized to a GE cell-cycle cDNA array filter. Both the levels of gene expression (Fig. 4C) and protein expression (Fig. 4D) of the cdk inhibitor p27kip1 were increased ~2.5-fold in TRAIL-treated T-cells compared with untreated cells. In contrast, the levels of gene expression of either p15INK4b (Fig. 4C) or p16INK4a, p18INK4c, p19INK4 days, and p21cip1 (Q.-S.M., C.M., T.L.D., unpublished observations) did not change significantly. p27kip1 can contribute to the association and activation of cyclin D with their complementary cdk and thereby mediate the arrest of cells at G1 phase of the cell cycle (31). Thus, the increased expression of p27kip1 observed here may be associated with the TRAIL-mediated arrest of cell cycle progression.

**TRAIL-induced anergy in diabeticogenic T-cells.** p27kip1 is important for the induction and maintenance of T-cell anergy. Uregulated p27kip1 results in defective IL-2 transcription and induced T-cell anergy, which can be rescued by exogenous IL-2 in vitro (32). To address whether TRAIL-upregulated p27kip1 is involved in the induction of anergy of diabeticogenic T-cells, we first determined the level of IL-2 production in diabeticogenic T-cells after stimulation by TRAIL. IL-2 production by diabeticogenic T-cells was significantly diminished after CD3 stimulation or CD3/CD28 costimulation in the presence of TRAIL (Fig. 5A). This TRAIL-induced suppression of T-cell proliferation was reversed upon CD3 stimulation of the diabeticogenic T-cells in the presence of exogenous IL-2 or sDR5 (Fig. 5B). Moreover, spleen T-cells from NOD mice treated in vivo with CY plus sDR5 exhibit higher proliferation (S.-E.L.-C., Y.H.C., unpublished observations) and elevated IL-2 production in response to the GAD65 islet autoantigen than T-cells from NOD mice treated with CY in the absence of sDR5 (Fig. 5C). Taken together, our results in Figs. 4 and 5 suggest that TRAIL may arrest NOD diabeticogenic T-cells at early G1 in the cell cycle by maintaining a higher level of expression of p27kip1, which may induce these T-cells to enter into a state of anergy.

**DISCUSSION**

In this study, we report that the blockade of endogenous TRAIL activity exacerbates autoimmune type 1 diabetes in NOD mice. Diabeticogenic T-cells from newly diabetic NOD mice treated with TRAIL in vitro undergo proliferative hyporesponsiveness to TCR stimulation rather than apoptosis. This hyporesponsiveness may be mediated by TRAIL-induced suppression of cell division, which is consistent with a previous report that TRAIL inhibits DNA synthesis and prevents the progression from G1 to S phase.
of lymphocytes in autoimmune arthritis in mice (15). Our finding further supports the notion that TRAIL is an immune regulator of autoimmune disease. It is noteworthy that TRAIL was also recently found to inhibit the proliferation of human autoantigen-specific T-cells without inducing apoptosis in vitro (29). Thus, blockade of endogenous TRAIL with sDR5 may eliminate this suppression, enhance the proliferative response of autoreactive effector T-cells to TCR stimulation, and exacerbate autoimmune type 1 diabetes. Interestingly, we also found that diabeticogenic T-cells are more sensitive to TRAIL-induced hyporesponsiveness compared with T-cells from 3-week-old NOD mice. This may be related to changes in gene expression profiles in T-cells during the development of type 1 diabetes (Q.-S.M., C.M., T.L.D., unpublished observations).

The mechanisms by which TRAIL suppresses the proliferation of autoreactive T-cells and downregulates an autoimmune response are unclear. One mechanism may involve the induction of anergy in autoreactive T-cells. Anergy was initially considered to be a mechanism of self-tolerance (33). However, anergizing TCR signals result in increased intracellular concentrations of the second messenger cAMP, which in turn upregulates cdk inhibitors such as p27kip1. These inhibitors sequester cyclin D2-cdk4 and cyclin E/cdk2 complexes and prevent progression of T-cells through the G1 restriction point of the cell cycle (33). The addition of IL-2 in the presence of an anergizing TCR signal leads to further degradation of p27kip1, prevention of anergy, and entry into S phase (33,34). Accordingly, we found that TRAIL can upregulate p27kip1 gene and protein expression in vitro as well as inhibit IL-2 production by diabeticogenic T-cells, and TRAIL blockade by sDR5 in vivo can increase IL-2 production by splenocytes in response to GAD65. Moreover, the proliferative hyporesponsiveness of diabeticogenic T-cells induced by TRAIL can be reversed by exposure to exogenous IL-2. Thus, TRAIL may induce the anergy of NOD diabeticogenic T-cells, which would be expected to elicit protection against type 1 diabetes. Although the mechanism by which TRAIL regulates the level of p27kip1 expression is unclear, a TRAIL-induced increase in p27kip1 protein expression may result from reduced p27kip1 degradation since diminished IL-2 production was observed in activated T-cells (Fig. 5A). The relative level of p27kip1 versus cdk4 is crucial in determining whether cdk4 is enzymatically active, and even small increases in the amount of p27kip1 are sufficient to suppress cdk activity (33). As TRAIL-induced hypoproliferation may contribute to the downregulation of cdk4 (29), further experimentation is necessary to test if the TRAIL-induced increase in p27kip1 leads to an association with its cdk targets in diabeticogenic T-cells.

Clonal deletion by apoptosis is considered to be a universal mechanism of self-tolerance. Although we and others (15,16,29) found that TRAIL, unlike FasL or TNF-α, did not induce the apoptosis of autoreactive T-cells, some leukocytes are susceptible to TRAIL-induced apoptosis in vitro. For example, TRAIL mediates CD4+ T-cell lysis of antigen-presenting macrophages (35). Specifically, TRAIL expressed by the D10 cloned Th2 line that lacks FasL and perforin expression can elicit apoptosis in macrophages, and this apoptosis is inhibitable by anti-TRAIL antibodies. Furthermore, while normal T-cells are resistant to TRAIL-induced apoptosis in vitro, T-cells from HIV-1–infected patients previously shown to exhibit increased CD95 sensitivity are more susceptible to TRAIL-induced cell death (36). TRAIL is also involved in dendritic cell–mediated apoptosis of activated human T-cells (11,12,37). In addition, TRAIL may play a role in negative selection in the thymus, as TRAIL can induce the apoptosis of immature CD4+CD8+ double positive human thymocytes stimulated in vitro by anti-CD3 (38).

Thus, TRAIL may participate in the maintenance of
macrophage homeostasis and the downregulation of central and peripheral immune responses. Whether this is the case in vivo remains to be determined. TRAIL-deficient mice on the C57BL6 and BALB/c genetic backgrounds do not show abnormalities in cell number in the bone marrow and spleen (13,14). However, these mice are susceptible to the development of autoimmune diseases, including STZ-induced diabetes and collagen-induced arthritis (27). In addition, these TRAIL knockout mice have enlarged thymuses, as well as severe defects in thymic negative selection and the intrinsic and extrinsic pathways of apoptosis. Macrophages from TRAIL knockout mice produce significantly higher amounts of IL-12 and nitric oxide (NO) than cells from wild-type mice in response to IFN-γ and/or LPS, and exhibit a reduced level of spontaneous apoptosis (S.-E.L.C., Y.H.C., unpublished observations). These findings indicate that TRAIL activity may regulate the development of central tolerance and indirectly diminish autoreactive T-cells via antigen-presenting cells. Since genetic epistatic effects can contribute to the severity of lymphoproliferative and autoimmune diseases, it will be important to backcross the TRAIL knockout phenotype onto the autoimmune NOD genetic background. Analyses of NOD.TRAIL−/− mice may uncover the normal physiological function of TRAIL during immunoregulation. Previously, we (19) and others (39–41) demonstrated that NK T-cell activation by the glycolipid α-galactosylceramide (α-GalCer) protects NOD mice from the onset of spontaneous and CY-accelerated type 1 diabetes. Interestingly, human Vα24+ NKT cells activated by α-GalCer express high levels of TRAIL (10), and NK cells from α-GalCer-treated mice also overexpress TRAIL (42). These results imply that TRAIL may be also involved in α-GalCer-stimulated protection against type 1 diabetes.

The findings discussed above may be incorporated into a model (Fig. 6) in which TRAIL may regulate immune responses in the thymus and periphery via different pathways to prevent the onset of autoimmune type 1 diabetes. In this manner, TRAIL may be involved in the maintenance of immune homeostasis and/or the induction of self-tolerance. TRAIL and TRAIL receptors are widely expressed in various tissues, including pancreatic islet β-cells (26,43,44). Our cDNA array analyses show that the gene expression of TRAIL is upregulated in pancreatic islets during the development of type 1 diabetes, as well as in TNF-α + IFN-γ-induced apoptotic Min6 cells. It is currently unknown how TRAIL is upregulated in islet β-cells undergoing apoptosis. Recent studies indicate that certain cytokines, including TNF-α and/or IFN-γ, can increase the expression of TRAIL by tumor cells and lymphocytes (45,46). Thus, TNF-α and/or IFN-γ may contribute to the elevated expression of TRAIL in cytokine-activated or apoptotic islet β-cells. Interestingly, we found that culture of freshly isolated pancreatic islets or Min6 cells with TRAIL does not induce islet β-cell apoptosis. This is consistent with the recent finding that TRAIL does not induce apoptosis in most freshly isolated human islets cells (43). Moreover, our data also showed that STZ-induced diabetes in TRAIL-deficient mice and NOD mice treated with CY plus sDR5 exhibit severe destructive insulitis compared with wild-type mice or NOD mice treated with CY only (27 and S.-E.L.C., Y.H.C., unpublished observations). Based on these results, we propose that islet β-cells may have a self-defense system that controls their survival by locally regulating autoimmune reactions mediated by TRAIL, whose expression is upregulated in cytokine (TNF-α + IFN-γ)-activated or apoptotic islet β-cells (Fig. 6). NOD mice may be critically dependent on the early TRAIL-induced defense mechanisms elicited by islet β-cells and/or immunocompetent cells (e.g., T-cells, NK T-cells and dendritic cells) to delay the onset of type 1 diabetes. Conversely, if this TRAIL-induced defense fails, NOD mice may succumb immediately to type 1 diabetes. It follows that genes expressed within the target tissue of certain organ-specific autoimmune diseases, e.g., type 1 diabetes, may influence the onset of inflammation and thereby controls disease susceptibility. For example, an islet β-cell antiviral defense appears to play a crucial role in the prevention of virus-induced type 1 diabetes (47), thus providing additional evidence for the existence of an islet β-cell self-defense system.

In summary, our observations show that TRAIL blockade can exacerbate autoimmune diabetes and that TRAIL can prevent autoreactive T-cell proliferation by inhibiting cell division and IL-2 production, possibly via the upregulation of the cdk inhibitor p27kip1. Although TRAIL expression is upregulated in cytokine-activated or apoptotic islet β-cells, TRAIL does not actually induce the apoptosis of islet β-cells. Thus, TRAIL may not contribute to pancreatic islet destruction. Rather, our data suggest that TRAIL is a potent immune regulator of type 1 diabetes and may accomplish this by inducing the anergy of diabetogenic T-cells and the apoptosis of thymocytes.

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TRAIL BLOCKADE EXACERBATES TYPE 1 DIABETES

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1974 DIABETES, VOL. 52, AUGUST 2003


