The B-Subunit of Cholera Toxin Induces Immunoregulatory Cells and Prevents Diabetes in the NOD Mouse

Douglas O. Sobel, Boris Yankelevich, Deepshikha Goyal, David Nelson, and Amitabha Mazumder

The B-subunit of the cholera toxin molecule (CT-B) has T-cell immunomodulatory properties. Because the pathogenesis of diabetes in the nonobese diabetic (NOD) mouse model of IDDM is thought to be a T-cell–mediated process due to an imbalance of immunoregulatory and anti-islet effector cells, we examined the effect of CT-B administration on the development of diabetes in the NOD mouse and assessed whether this potential diabetes-sparing effect of CT-B is mediated by changes in immunoregulatory and/or anti-islet cytotoxic effector cell activity. The administration of either intravenous or intraperitoneal CT-B decreased the development of diabetes with no apparent drug toxicity. At 6 months of age, only 18% of CT-B vs. 75% of saline-treated animals had diabetes. Histopathological examination revealed less islet atrophy in CT-B–treated animals. The in vitro proliferative responses of mononuclear splenocytes and thymocytes to concanavalin A and lipolysaccharide and the proportion of B-cells and T-cell subsets were not altered by CT-B treatment. CT-B administration did not inhibit the primary immunization of mice to tetanus toxoid. The development of diabetes in irradiated NOD mice was slower in the animals injected with spleen cells (SC) from CT-B–treated than from saline-treated NOD mice, suggesting that CT-B decreases anti-islet effector cell activity. The injection of SC from CT-B–treated mice inhibited the adoptive transfer of diabetes by SC from diabetic mice into irradiated NOD mice, documenting that CT-B administration induces regulatory cell activity. In conclusion, CT-B administration prevents the development of diabetes in NOD mice by inhibiting the immune destruction of islets. This islet-sparing activity appears mediated, at least in part, by the induction of regulatory cells and, in turn, suppression of anti-islet effector cells, which is not associated with generalized immunosuppression or T- or B-cell depletion. *Diabetes* 47:186–191, 1998

From the Department of Pediatrics (D.O.S., D.G., D.N.) and the Lombardi Cancer Research Center (B.Y., A.M.), Bone Marrow Transplant Program, Georgetown University School of Medicine, Washington, DC.

Address correspondence and reprint requests to Douglas O. Sobel, MD, Division of Pediatric Endocrinology and Metabolism, 3800 Reservoir Rd., N.W., Washington, DC 20007-2197.

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ConA, concanavalin A; CT-B, cholera toxin B-subunit; CT-B-SC, CT-B spleen cells; D-SC, diabetic spleen cells; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; H&E, hematoxylin and eosin; LPS, lipopolysaccharide; Sal-SC, saline spleen cells; SC, spleen cells.

The nonobese diabetic (NOD) mouse is an animal model for human IDDM (1,2). Both disorders are due to an autoimmune process characterized by lymphocytic infiltration of pancreatic islets (3,4), the presence of serum islet cell antibodies (5,6), and the importance of cell-mediated immunity in the development of diabetes (7–9). Mononuclear splenocytes and, specifically, T-cells from NOD mice have also been demonstrated to adoptively transfer diabetes into NOD mice (10). The role of T-cells in the pathogenesis of diabetes in NOD mice is further underscored by the predominance of T-cells within the inflamed islets of NOD mice (11) and the prevention of diabetes in NOD mice after neonatal thymectomy (12) or the administration of either anti-CD3 or anti-CD4 antibody (13,14). It is thought that a defect in the immunoregulatory cells of NOD mice plays a permissive role in the development of spontaneous diabetes and the adoptive transfer of diabetes in NOD mice (15).

Although immunosuppression with specific drugs that alter T-cell immune responses, such as cyclosporin and FK506, can inhibit the development of diabetes in NOD mice and humans (16,17), the development of treatments without the induction of a general immunosuppressive state would be safer and more useful.

The B-subunit of the cholera toxin molecule (CT-B) is the binding subunit of the toxin, is nontoxic (18–20), and possesses immunomodulatory properties that potentially could inhibit the autoimmune process of IDDM. This compound has been demonstrated to alter T-cell–mediated responses (21,22). In addition, we have recently reported that CT-B induces donor-specific tolerance to allogeneic bone marrow (23) and prevents the induction of acute graft versus host disease (24).

In this study, we tested the hypotheses that the administration of CT-B inhibits the development of islet destruction and diabetes in the NOD mouse and that this potential inhibition is mediated by a suppression of anti-islet effector cell activity and the induction of immunoregulatory cell activity.

RESEARCH DESIGN AND METHODS

Mice. NOD mice were obtained from Taconic Farms (Germantown, NY).

CT-B treatment protocol. CT-B was purchased from List Biological Laboratories (Campbell, CA). CT-B was prepared from cholera toxin isolated from supernatant of *Vibrio cholera*. Preparations of CT-B contained very low levels of endotoxin (<2 pg/dose). Injections of 1 pg endotoxin (Cape Cod Associates) i.p. three times a week for 4 weeks to female NOD mice (n = 7) did not decrease the development of diabetes as compared with saline-treated (n = 8) control mice (data not shown). The final incidences of diabetes in saline- and endotoxin-treated mice were 62.5 and 75%. To determine the effect of CT-B administration on the development

of diabetes, 6- to 7-week-old female NOD mice were randomly placed into three groups (8 mice/group). One group received isotonic saline intraperitoneally three times a week for 4 weeks, another group received 10 μg CT-B i.p. three times a week for 4 weeks, and the last group received 10 μg i.v. weekly for 4 weeks.

Adoptive transfer experiments to assess anti-islet effector cell activity. The effect of CT-B on anti-islet effector cell activity was studied by comparing the development of diabetes in irradiated syngeneic mice administered mononuclear spleen cells (SC) from CT-B–treated and saline-treated NOD mice. At 6 weeks of age, female NOD mice were treated with either saline or 10 μg CT-B i.p. three times a week for 4 weeks. One week after the cessation of treatments, the animals were killed and mononuclear leukocytes were isolated from dissected spleens by Ficoll gradient centrifugation (Ficoll/Lite-Lympholl, Atlantic Biologicals, Norcross, GA). Mononuclear SC from similarly treated animals were pooled and intravenously injected (4×10^7 cells/recipient) into 10-week-old γ-irradiated (750 rads) female NOD recipient mice (8 recipient mice per treatment group). The rates of development of diabetes were compared in animals receiving cells from saline-treated and CT-B–treated animals.

Adoptive transfer experiments to assess immunoregulatory cell activity. The hypothesis that the inhibitory action of CT-B on the diabetic process is mediated by the induction of immunoregulatory cells was studied in experiments to determine whether the administration of SC from CT-B–treated mice inhibit the adoptive transfer of diabetes in NOD mice (25). Ten-week-old male NOD recipient mice were γ irradiated (750 rads) and randomly placed into the following treatment groups (10 mice per group): group 1 = irradiation alone; group 2 = diabetic spleen cells (D-SC); group 3 = saline spleen cells (Sal-SC) + D-SC; group 4 = cholera toxin B-subunit spleen cells (CT-B-SC) + D-SC.

Within 3 h of irradiation, mice in groups 3 and 4 received 10^8 mononuclear SC by intravenous infusion from 10- to 11-week-old female NOD mice previously administered either saline or CT-B (10 μg i.p. three times a week) 1 week after the last CT-B or saline injection. The day after irradiation, 2×10^7 mononuclear SC from acutely diabetic female NOD mice were injected intravenously into mice in groups 2, 3, and 4. Animals receiving D-SC (group 2) and no cells (group 1) served as positive and negative controls, respectively. The development of diabetes was then compared in recipient animals.

Experiment to assess the induction of primary immunization. At 5 weeks of age, female NOD mice were injected with either 10 μg CT-B ($n = 4$) or saline ($n = 4$), i.p. three times a week for 6 weeks. After 4 weeks, mice were injected intramuscularly with 0.1 LF units tetanus toxoid absorbed to alum. Mononuclear SC were isolated 10 days later from all mice and tested for proliferative reactivity to tetanus antigen using a standard thymidine incorporation test. SC (10^5 cells/well) were incubated for 5 days with tetanus toxoid (10 μg/ml, 1,557 LF units/mg) or complete RPMI 1640 media alone. ^{3}H-thymidine (0.5 μCi/well) was then added to cells, which were then harvested 16 h later and counted for radioactivity. The stimulation index was calculated as $CPM_{tetanus} \div CPM_{media}$.

Assessment and diagnosis of diabetes. Blood glucose was tested weekly by reflectometer (Accucheck III, Boehringer Mannheim, Indianapolis, IN). Animals were diagnosed with diabetes once the blood glucose remained over 14 mmol/l over 2 consecutive days.

Histological examination. In another experiment, the effect of CT-B treatment on pancreatic histopathology was assessed in 12- and 26-week-old NOD mice. At 12 weeks of age, 1 week after the cessation of treatment, 5 nondiabetic CT-B–treated mice (10 μg i.p. three times a week for 4 weeks) and 5 nondiabetic saline-treated control mice were killed. Later, at 26 weeks of age, 3 intraperitoneally CT-B–treated mice (all nondiabetic) and 4 saline-treated mice (3 diabetic and 1 nondiabetic) were killed. Each pancreas was dissected, fixed in buffered formalin, embedded in paraffin, cut into 5-μm sections, and stained with hematoxylin and eosin (H&E). Islets from each section were blindly assessed for the degree of insulitis and islet destruction and for number of islets per low power field (150×). The histopathological score of each islet was determined as follows: 0 points for no inflammation, 1 point if 0–10% of the islet is inflamed, 2 points for 10–25% inflammation, 3 points for 25–50% inflammation, and 4 points for over 50% inflammation, extensive islet degranulation or fibrosis, or islet atrophy. For each animal, the number of islets per low power field (150×) was counted in more than 20 fields from at least three different sections.

Immunohistochemical staining for insulin. The pancreases of 12- and 26-week-old CT-B–treated and control age-matched mice were assessed for the degree of islet insulin staining. Formalin-fixed paraffin-embedded pancreatic sections (5 μm) were cut and immunohistochemically stained with an immunoperoxidase technique (avidin biotin complex method; Vectastain, Vector Laboratories, Burlington, CA) using 3-diaminobenzidine as chromagen. Guinea pig anti-bovine insulin antibody (Linco, St. Louis, MO), which cross-reacts with mouse insulin, was used as the primary antibody, and biotin-conjugated donkey anti-guinea pig antibody (Jackson ImmunoResearch, West Grove, PA) was used as the secondary antibody. After immunostaining, the tissues were counterstained with hematoxylin. The proportion of the islet stained with insulin was scored in each islet as follows: 5 points for over 75% of the islet stained, 4 points for 50–75% staining, 3 points for 25–50%, 2 points for 10–25%, 1 point for 1–10%, and 0 points for under 1% staining.

Cell preparations. One week after the cessation of treatments, spleens were harvested and single cell suspensions were prepared by mechanical disruption from 5 nondiabetic control mice and 5 nondiabetic intraperitoneally CT-B–treated mice. Mononuclear leukocytes were then isolated by Ficoll gradient centrifugation (Ficol/Lite-Lympholl, Atlantic Biologicals) and individually assessed for proliferative responses to concanavalin A (ConA) and lipolysaccharide (LPS). Concomitantly, thymii from 5 CT-B–treated and 5 saline-treated nondiabetic NOD mice were dissected and mechanically disrupted to single cell suspensions. Isolated thymocytes from each mouse were tested for proliferative response to ConA.

Cell proliferation assays. One hundred thousand cells per well ($n = 4$) were incubated with ConA (5 μg/ml, Sigma, St. Louis, MO) or LPS (5 μg/ml, Sigma) in complete media or in complete media alone (RPMI 1640 supplemented with 10% fetal calf serum [FCS]) (Gibco-BRL, Grand Island, NY), 2 mmol/l L-glutamine (Gibco-BRL), 10^{-5} mol/l 2-mercaptoethanol (Sigma), 2 mmol/l nonessential amino acids (Gibco-BRL), 25 mmol/l HEPES buffer (Gibco-BRL), 100 μg/ml penicillin, and 100 μg/ml streptomycin (Gibco-BRL). After 5 days of incubation in 5% pCO_2 at 37°C, the cells were pulsed with 1 μCi of ^{3}H-thymidine (Du Pont–NEN, Boston, MA), harvested 16 h later onto filter paper, and counted for radioactivity in a β-scintillation counter. The stimulation index was calculated to $CPM_{mitogen} \div CPM_{media}$.

Flow cytometric analysis. Mononuclear leukocytes were isolated from spleens 1 week after the cessation of treatment and incubated for 30 min with the following antibodies obtained from Gibco-BRL: fluorescein isothiocyanate (FITC)-conjugated B220 (pan B-cell), phycoerythrin-conjugated anti-CD8 (cytotoxic/suppressor phenotype), and FITC-conjugated anti-CD4 (helper/inducer phenotype). A minimum of 10,000 cells were analyzed by flow cytometry (FacStar, Becton Dickinson, Mountain View, CA).

Statistical analysis. The product-limit method of Kaplan and Meier estimated survival function (from diabetes) in treatment groups. Gehan's Wilcoxon test compared the product-limit function. Group means were compared by Student's t test.

RESULTS

CT-B administration prevents the development of diabetes in NOD mice. CT-B administered by either intravenous or intraperitoneal route decreased the development of diabetes as assessed by survival (from diabetes) curve analysis ($P < 0.03$ and $P < 0.02$, respectively) (Fig. 1). At 6 months of age, only 18.8% of all CT-B–treated NOD mice vs. 75% of saline-treated mice ($P < 0.01$) had diabetes. After 6 months of age, 6 nondiabetic intravenously CT-B–treated and 4 nondiabetic intraperitoneally CT-B–treated mice were observed for the development of diabetes for another 2 months. Nine of ten animals remained diabetes free.

CT-B causes no alterations in weight gain or activity. Before the onset of diabetes, animals from each group gained weight similarly. CT-B did not cause diarrhea,

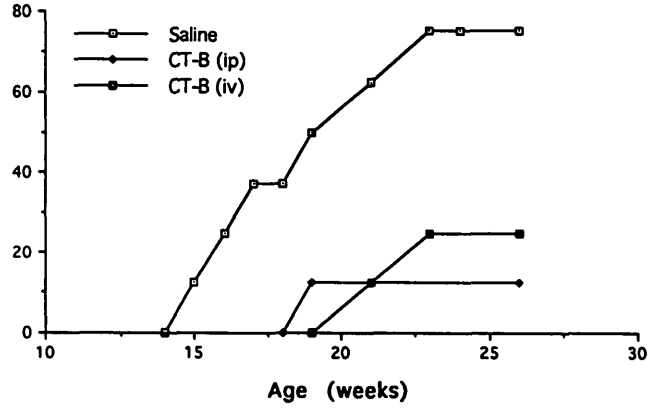

FIG. 1. Incidence of diabetes in NOD mice administered CT-B (10 μg i.v.) each week for 4 weeks ($n = 8$), CT-B (10 μg i.p.) three times a week for 4 weeks ($n = 8$), and saline (10 μg i.p.) three times a week for 4 weeks ($n = 8$) beginning at 6–7 weeks of age.

lethargy, scruffiness, or abnormal activity, such as hunching. At 5 months of age, the mean weights (± SE) of saline-treated (n = 5), intravenously CT-B–treated (n = 8), and intraperitoneally CT-B–treated (n = 7) nondiabetic mice were 26.8 ± 1.0, 26.5 ± 1.4, and 25.6 ± 1.1 g, respectively.

Histopathological islet scores are greater in CT-B–treated mice. Histological examination of H&E–stained pancreases revealed a predominantly mononuclear inflammatory response within islets, particularly in the saline-treated mice. The exocrine tissue was overall spared from the inflammatory response in all groups of mice. In 12-week-old mice, the mean islet inflammatory score of CT-B mice (n = 5) was 25% lower than that found in the control mice (n = 5) but did not achieve statistical significance (Fig. 2*A*). As the disease progressed, the mean islet histopathological score in 26-week-old mice was 47% lower in CT-B–treated (n = 3) than in saline-treated 26-week-old animals (n = 4) (P < 0.02) but was no different from the mean histopathological score in the 12-week-old CT-B–injected mice (Fig. 2*A*). The mean number (SE) of islets per low power field was significantly greater (P < 0.01) in 12-week-old CT-B–treated mice (2.5 ± 0.4 vs. 0.8 ± 0.3) vs. control mice. At 26 weeks, the mean number of islets in CT-B–treated mice (n = 3) was 1.5 ± 0.4 as compared with only 0.6 ± 0.3 in saline-treated mice (n = 4). However, these means were not significantly different.

Immunohistochemical staining of islets for insulin in pancreases of 12- and 26-week-old CT-B– and saline-treated control mice was examined (Fig. 2*B*). In 12-week-old mice, the insulin-staining scores of CT-B–treated mice (n = 5) and control animals (n = 5) were similar. The mean insulin-staining score was four times greater in 26-week-old CT-B–treated mice (n = 3) than in control mice (n = 4) (P < 0.02). The mean insulin-staining scores of 12- and 26-week-old CT-B–injected mice were similar (Fig. 2*B*). Insulin staining was not found in areas of intense inflammation in either treatment group.

Thymocyte and mononuclear splenocyte proliferative responses to mitogens are unaltered by CT-B. To determine whether CT-B induces a state of generalized immunosuppression, the proliferative responses of mononuclear splenocytes to ConA and LPS were compared in CT-B– and saline-treated NOD mice 1 week after the cessation of treatments. The proliferative responses to ConA and LPS were similar in the saline (n = 5) and intraperitoneally CT-B–treated mice (n = 5) (Fig. 3). Thymocyte activation by ConA was compared in saline-administered (n = 4) and CT-B–administered (n = 3) NOD mice 1 week after the cessation of treatment. ConA induced similar proliferative responses in thymocytes from saline- and CT-B–treated animals with mean stimulatory indexes (± SE) of 5.3 ± 0.9 and 4.0 ± 0.3, respectively.

CT-B does not change the proportion of T-cell subsets or B-cells. Mononuclear SC phenotypes were assessed 1 week after the cessation of treatment. The proportion of $B220^+$ cells, $CD4^+$ cells, and $CD8^+$ cells were similar in the intraperitoneally CT-B– and saline-treated groups (Table 1).

CT-B does not suppress the induction of primary immunization to tetanus toxoid. To study the possibility that CT-B causes a generalized immunosuppressive state, mononuclear SC proliferative response to tetanus antigen after primary immunization was assessed. Mononuclear SC proliferative responses (stimulation indexes) to tetanus toxoid were similar in saline- and CT-B–administered mice (7.3 ± 0.4 vs. 6.1 ± 1.2 in control mice).

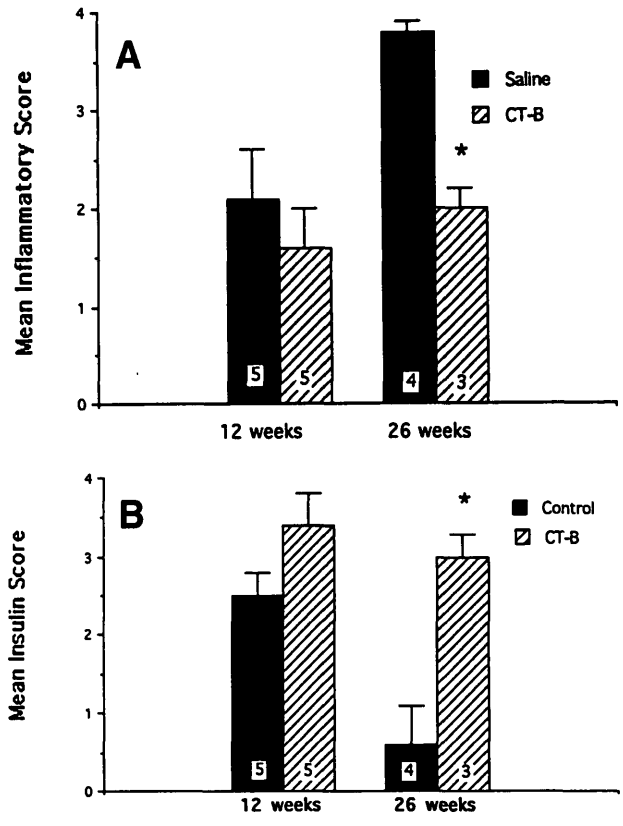

FIG. 2. ***A*: The mean histopathological scores (± SE) of 12- and 26-week-old NOD mice injected with CT-B (10 µg i.p. three times a week) and saline. *P < 0.02 vs. saline-treated control mice.** ***B*: The mean insulin-staining scores (± SE) of 12- and 26-week-old NOD mice treated with CT-B (10 µg i.p. three times a week) and saline. *P < 0.02 vs. saline-treated control mice.**

CT-B administration decreases splenocyte anti-islet effector cell activity. The effect of CT-B administration on splenocyte anti-islet effector cell activity was examined by comparing the development of diabetes in irradiated recipient mice (8 mice per group) receiving splenocytes from CT-B– and saline-treated animals. The rate of diabetes development was significantly slower in mice administered splenocytes from CT-B–treated animals by survival curve analysis (P < 0.05) (Fig. 4).

CT-B induces immunoregulatory cells that inhibit the adoptive transfer of diabetes. The role of immunoregulatory cells in the mechanism of CT-B inhibition of diabetes was studied by comparing the development of diabetes in irradiated male NOD mice injected with CT-B-SC + D-SC with mice injected with Sal-SC + D-SC (Fig. 5). The development of diabetes in NOD mice injected with CT-B-SC + D-SC was significantly decreased as compared with mice administered S-SC + D-SC by survival (from diabetes) curve analysis (P < 0.02). Fourteen weeks after cell transfer, only 20% of the CT-B-SC + D-SC–treated mice as compared with 80% of the Sal-SC + D-SC–treated mice developed diabetes (χ^2 = 6.7, P < 0.01). In fact, the development of diabetes in the CT-B-SC + D-SC group was similar to that found in mice receiving irradiation only.

DISCUSSION

This is the first report demonstrating that the administration of small doses of CT-B prevents the development of autoim-

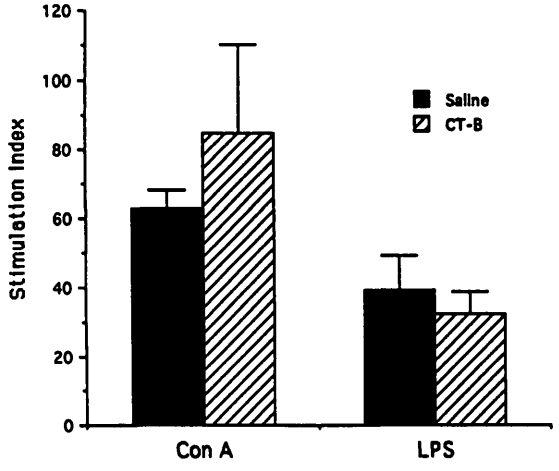

FIG. 3. The proliferative responses to ConA and LPS of mononuclear splenocytes from NOD mice administered CT-B (10 μg i.p. three times a week [n = 5]) and saline (n = 5) for 4 weeks. See CELL PROLIFERATION ASSAYS for explanation. For each mitogen, the mean stimulation index of cells from CT-B– and saline-administered mice were not significantly different.

mune diabetes. Moreover, CT-B administration has not previously been found to inhibit any autoimmune disorder. Both routes of CT-B administration, intravenously and intraperitoneally, potently decreased the development of diabetes. The diabetes-sparing effect of CT-B was remarkably long lasting. Most (75%) of the i.v. CT-B–treated animals remained diabetes-free at the end of the observation period at 8 months of age. Almost all (95%) of the CT-B–treated animals that were diabetes-free at 6 months of age never developed diabetes.

Histopathological examination of the pancreas revealed that the diabetes-sparing action of CT-B is due to an inhibition of the autoimmune destruction of islets and the sparing of insulin-secreting β-cells. This action of CT-B occurs as early as 1 month after the initiation of treatment. Moreover, the islet-sparing effect of CT-B continued after the cessation of treatment, since the degree of islet insulin staining and immune destruction was not altered from 12 to 26 weeks in CT-B–treated animals. All areas of intense islet mononuclear cell infiltration were associated with a lack of islet insulin staining, signifying areas of β-cell dysfunction or death. This suggests the preponderance of anti-islet effector cells in these areas and does not represent areas of immunoregulatory cells.

Cyclosporin and other immunosuppressive therapies have been demonstrated to inhibit the diabetic process in humans (8). We found no support that CT-B induces a state of generalized immunosuppression. Proliferative responses to both T-cell mitogen (ConA) and B-cell mitogen (LPS) were not reduced in splenocytes from CT-B–treated mice. Furthermore, CT-B did not inhibit the induction of primary immunization to tetanus antigen.

Flow cytometric analysis of splenocytes revealed that the diabetes-sparing action of CT-B is not due to the depletion of either B-cells ($B220^+$ cells) or T-cell subsets. Thus, there was no evidence that the diabetes-sparing activity of CT-B is mediated by selective $CD8^+$ T-cell depletion, an effect of CT-B that we have previously observed in murine T-cells in vitro (26). Moreover, flow cytometric analysis demonstrated that the diabetes-sparing effect of CT-B was not mediated by the preferential induction of a mononuclear leukocyte subset.

TABLE 1
Phenotype of mononuclear splenocytes from saline control and CT-B–injected NOD mice 1 week after the last injection of 10 μg CT-B

	n	$B220^+$	$CD4^+$	$CD8^+$
Control mice	3	30.2 ± 1.0	32.52 ± 1.4	13.7 ± 1.1
CT-B mice	3	34.6 ± 1.3	31.4 ± 1.2	12.7 ± 0.8

Data are means ± SE.

To explore the mechanism of the diabetes-sparing effect of CT-B, we studied the effect of CT-B administration on splenocyte anti-islet cytotoxic activity and immunoregulatory cell activity. The development of diabetes in irradiated NOD mice was decreased in animals receiving SC from CT-B–treated mice as compared with irradiated mice receiving cells from saline-treated NOD mice. These data support the hypothesis that the diabetes-sparing effect of CT-B treatment is mediated, at least in part, by depressed anti-islet effector cell activity.

The depressed anti-islet effector cell activity described above may be due to a direct decrease in effector cell number or activity by CT-B or to an induction of immunoregulatory cells. Therefore, the hypothesis that CT-B induces immunoregulatory cells that are able to mediate the inhibition of effector cells and, in turn, the diabetic process was tested. Our results document that the adoptive transfer of diabetes by D-SC infusion was inhibited in mice administered SC from CT-B–treated animals compared with mice injected with SC from saline-treated mice. Moreover, cells from CT-B–treated mice suppressed the development of diabetes induced by the injection of D-SC to the level observed in mice receiving no D-SC. These results strongly support the hypothesis that CT-B treatment inhibits anti-islet effector activity and diabetes via an induction of immunoregulatory cells. This induction of immunoregulatory cells by CT-B has not heretofore been reported in any animal model. Furthermore, our data demonstrate that CT-B treatment not only prevents the development of anti-islet effector activity and diabetes but also can

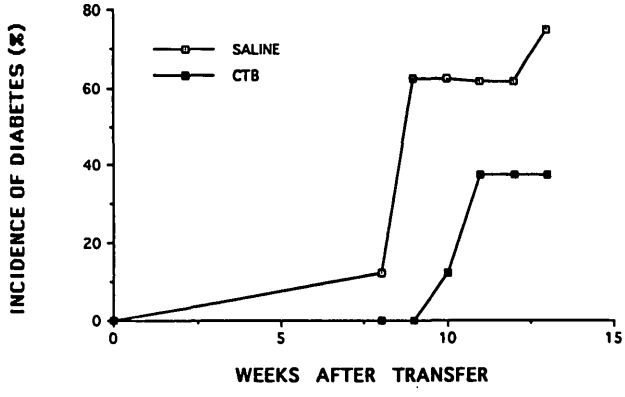

FIG. 4. The effect of CT-B administration on anti-islet effector cell activity. Mononuclear splenocytes (4×10^7 cells) from CT-B–treated (10 μg i.p. three times a week) and saline-treated NOD female mice were each intravenously administered to two groups (n = 8 per group) of irradiated (750 rads) 10-week-old NOD mice. The development of diabetes was then compared in the two groups of irradiated mice and found to be lower in mice injected with cells from CT-B–treated animals ($P < 0.05$).

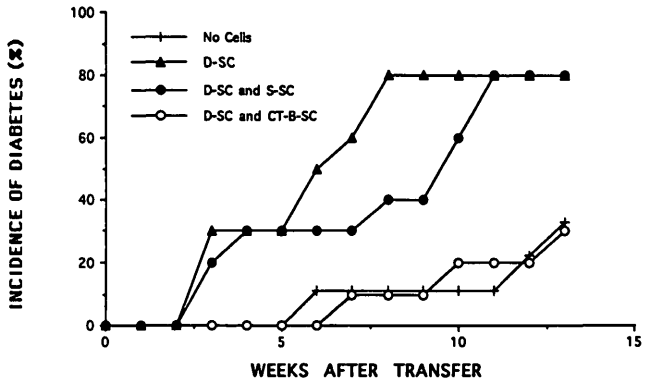

FIG. 5. Protection against the adoptive transfer of diabetes with SC from CT-B–treated mice. The development of diabetes in irradiated NOD mice (n = 10) infused with D-SC + CT-B-SC was compared with the development of diabetes in irradiated NOD mice (n = 10) treated with D-SC + Sal-SC. Irradiated animals treated with D-SC alone (n = 10) and no SC (irradiated alone) (n = 10) served as positive and negative controls. The rate of development of diabetes was lower in animals infused with D-SC + CT-B-SC than those infused with D-SC + Sal-SC (P < 0.02).

inhibit the already activated anti-islet effector cells present in D-SC. This inhibition on activated cells has significant implications on the possible therapeutic role of CT-B.

Disease-specific immunoregulatory cells have been found in NOD mice. The infusion of T-cell clones isolated and propagated from NOD islets prevents spontaneous disease (27) and suppresses accelerated diabetes in adoptive transfer experiments (28). Yet, these endogenously found T-cells do not prevent disease in NOD mice. In fact, immunoregulatory cells are thus thought to be defective in the NOD mouse (15). This defect may, in turn, play a role in the pathogenesis of diabetes. Thus, it is possible that CT-B administration reverses the imbalance of effector/regulatory cells thought to cause diabetes.

The induction of immunoregulatory cells with suppressed development of diabetes has been previously demonstrated in the NOD mouse after the administration of two similar substances that augment immune responses, i.e., Freund's adjuvant (29) and bacille Calmette-Guérin (30). The regulatory cells induced by Freund's adjuvant may not be similar to the population of regulatory cells induced by CT-B, since Freund's adjuvant causes a transient generalized immunosuppression (31), which is a state not found in CT-B–treated mice. The identity of the immunoregulatory cell(s) induced by CT-B has yet to be identified.

Thymic dysfunction in the NOD mouse is thought to play a role in the pathogenesis of diabetes (12,28,32). Furthermore, thymic T-cell anergy, manifested in part by thymocyte unresponsiveness to ConA, is thought to be related to susceptibility of the NOD mouse to diabetes (32). Reversal of this anergy could explain how CT-B depresses anti-islet effector cell activity and decreases the incidence of diabetes. However, the mean proliferative response to ConA of thymocytes from saline-control and CT-B–treated animals was similar, suggesting that the induction of immunoregulatory cells and the prevention of diabetes by CT-B is not mediated by the reversal of the thymic dysfunction present in NOD mice (29). In contrast, the administration of Freund's adjuvant to NOD mice enhanced the ability of thymocytes to proliferate in response to ConA (29). This further suggests that the mechanism of diabetes prevention by CT-B is different from that induced by immunostimulants. There are many immunological properties of CT-B that differ from those of Freund's adjuvant, which may explain its diabetes-sparing activity. Properties unique to CT-B and not Freund's adjuvant include the induction of T-cell apoptosis (26), the direct suppression of antigen and ConA activation (21,22), and the binding and stimulation of GM_1 ganglioside (33), which has been demonstrated to have immunomodulatory properties (34).

In conclusion, the administration of CT-B potently decreased the development of islet destruction and diabetes in NOD mice. The mechanism of this long-lasting CT-B action appears to be mediated by the suppression of anti-islet effector activity, which is due to the induction of immunoregulatory cells. Future studies exploring the diabetes-sparing activity of this novel immunomodulator may lead to a better understanding of the pathogenesis and treatment of IDDM.

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