α-Interferon Inhibits the Development of Diabetes in NOD Mice

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The NOD mouse is a model of human IDDM, which is characterized by a cell-mediated autoimmune process resulting in spontaneous diabetes. α-Interferon (IFN-α) is thought to play a pathogenic role in this autoimmune process. We report that recombinant α-interferon (rIFN-α) administration decreases the development of spontaneous diabetes and the passive transfer of diabetes in NOD mice. Spontaneous diabetes was inhibited by IFN-α in a dose-dependent fashion. A dose of as little as 20×10^3 U inhibited diabetes development, while a dose of 100×10^3 U potently prevented diabetes (14% incidence vs. 70% incidence in control mice). Even at the termination of the experiment, nondiabetic mice administered rIFN-α maintained normal glucose tolerance. Islet inflammation was 65% lower in the pancreases of rIFN-α mice. rIFN-α administration decreased anti-islet effector cell bioactivity of spleen cells without inducing generalized immunosuppression. Passive transfer experiments demonstrated that the decreased anti-islet effector cell activity was not a direct action of rIFN-α on these cells. In conclusion, rIFN-α potently and paradoxically prevents diabetes by indirectly decreasing anti-islet effector cell activity and in turn the development of insulitis without inducing generalized immunosuppression. This work, which goes against our current understanding of the role of rIFN-α in autoimmunity, may have significant implications to further our understanding of the pathogenesis of IDDM and to further the development of novel modes to prevent the disease.
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The NOD mouse is a model of IDDM, which is characterized by an autoimmune process resulting in spontaneous diabetes (1,2). As in human IDDM, insulitis, elevated serum islet autoantibodies, and autoimmune involvement of other endocrine organs are found in the NOD mouse (3–5). In addition, the importance of T-cells in the pathogenesis of autoimmune diabetes in the NOD mouse and people with IDDM is underscored by the inhibition of the diabetic process after treatments that inhibit T-cell function (6,7).

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Con A, concanavalin A; IFN, interferon; LCMV, lymphocitic choriomeningitis virus; LF, limits of flocculation; MHC, major histocompatibility complex; rIFN, recombinant interferon.

α-Interferon (IFN-α) is produced by immune cells as well as most other cells and has important immunological properties (8). IFN-α affects the function of T-cells (9) as well as macrophages (10) and B-cells (11) and has been shown to increase autoimmune processes. The administration of IFN-α accelerates autoimmune disease in NZB/W mice (12). In addition, IFN-α is thought to play a pathogenic role in the development of diabetes, since transgenic mice expressing IFN-α within islets develop diabetes (13). Also, IFN-α mRNA is present in the islet cells in the BB rat model of IDDM (14), and immunoreactive IFN-α and IFN-α mRNA have been found in β-cells (15) and whole pancreas (16), respectively, of people with IDDM. However, recent viral studies suggest a paradoxical opposite effect of IFN-α. Infection of both the NOD mouse and BB rat models of IDDM with lymphocitic choriomeningitis virus (LCMV) inhibits the development of diabetes (17,18). Because viruses are potent inducers of IFN-α (19), this cytokine may be important in mediating the diabetes-sparing effect of LCMV. Indeed, we recently reported that IFN-α administration inhibits the development of diabetes in BB rats (20). The present study examines the hypothesis that IFN-α mediates the diabetes sparing-effect of LCMV and determines whether the administration of recombinant α-interferon (rIFN-α) to NOD mice inhibits the development of insulitis and diabetes. In addition, the effect of IFN-α administration on the development of anti-islet effector activity and generalized immunosuppression was assessed.

RESEARCH DESIGN AND METHODS

Animals. NOD mice were purchased from Taconic Farms (Germantown, NY). Mice were housed in laminar flow hoods, and bedding, food, and water were autoclaved before use. Animals were provided food and water ad libitum. Viral serologic testing of blood from sentinel animals was performed routinely and remained negative.

Interferon. rIFN-α A/D bgl II (specific activity 50×10^6 U/mg) was obtained from Hoffman-La Roche (Little Falls, NJ) (21). This hybrid interferon recognizes and activates murine IFN-α receptors. Injections of rIFN-α and saline were administered intraperitoneally three times a week beginning at 5–6 weeks of age. A very low amount of endotoxin is present in the rIFN-α preparation (0.01–0.03 ng/10^8 U). Injections of 0.1 pg endotoxin (Associates of Cape Cod, Falmouth, MA), the amount contained in 10^5 U rIFN-α administered intraperitoneally three times a week, did not delay or inhibit the development of diabetes (data not shown).

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

Analysis of glucose tolerance. Glucose tolerance was assessed in four nondiabetic rIFN-α–treated 32-week-old NOD mice. After an overnight fast from food, mice were injected intraperitoneally with 0.5 mg glucose/g body wt using a 30 mg/ml glucose solution in water (16). Tail vein blood was obtained just before and 30, 60, and 120 min after the glucose injection and was assayed for glucose.

Histological examination. After death, pancreases were immediately dissected and fixed in buffered formalin. Paraffin-embedded tissues were cut into 5-μm sections and stained with hematoxylin and eosin. Two or more sections from each

pancreas were assessed and scored for the degree of insulitis in a blinded manner: 0 points for no islet inflammation, 1 point for 1–10% inflammation, 2 points for 10–25% inflammation, 3 points for 25–50% inflammation, and 4 points for over 50% inflammation, extensive islet degranulation, fibrosis, or islet atrophy. The average insulitis score for each animal was assessed. The mean of the average scores from saline- and rIFN-α–treated mice was compared.

Adoptive transfer experiments to assess anti-islet effector cell activity. The effect of rIFN-α administration on anti-islet effector cell activity was studied by comparing the development of diabetes in irradiated syngeneic mice administered mononuclear spleen cells from rIFN-α and saline-treated NOD mice. At 6 weeks of age, female NOD mice were treated with either saline or 10^5 U rIFN-α administered intraperitoneally three times a week for 4 weeks. Two weeks after the cessation of treatments, the animals were killed, and mononuclear leukocytes were isolated from dissected spleens by Ficoll gradient centrifugation. Mononuclear spleen cells 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 (eight recipient mice per treatment group). The rates of development of diabetes were compared in animals receiving cells from saline and rIFN-α–treated animals.

Examining the effect of rIFN-α on the passive transfer of diabetes. Male NOD mice were intraperitoneally administered either saline (n = 8) or 10^5 U rIFN-α (n = 8) three times a week for 5 weeks. Animals were then irradiated (750 rads) and intravenously administered mononuclear spleen cells (10^7) from acutely diabetic NOD mice. The rates of diabetes development were then compared.

Assessment of rIFN-α administration on the induction of primary immunization. Five-week-old female NOD mice were intraperitoneally administered either saline (n = 4) or rIFN-α (n = 4) three times a week for 6 weeks. After 4 weeks of treatment, all mice were injected intramuscularly with 0.1 limits of flocculation (LF) U of tetanus toxoid absorbed to alum. Ten days later, mononuclear spleen cells were isolated using Ficoll hypaque centrifugation and tested for reactivity to tetanus antigen using a standard thymidine incorporation test. Cells (10^5 cells/well) were incubated for 5 days with tetanus toxoid (2 μg/ml, 1,557 LF U/mg) or complete RPMI 1640 media alone. Cells were then incubated with [^{3}H]thymidine (0.5 μCi/well), harvested 16 h later, and counted for radioactivity.

Determining the ability of in vitro rIFN-α to alter spleen cell modulation of T-cell responses. Mononuclear spleen cells (10^{-5} cells/0.2 ml well) from untreated 5- to 8-week-old NOD and Balb/c mice were incubated for 2 days with rIFN-α at doses of 0, 2, 20, 200, and 2,000 μg/well in complete RPMI 1640 media. Cells were then washed, irradiated (1,500 rads), and incubated with concanavalin A (Con A) (1 μg/ml) and syngeneic mononuclear spleen cells, serving as responder cells, at a test cell:responder cell ratio of 3:1. The responder cells were never incubated with rIFN-α. After 72 h, [^{3}H]thymidine (0.5 μCi) was added to each well, and the cells were harvested 16 h later and counted for radioactivity.

Statistical analysis. The product-limit method of Kaplan and Meier was used to estimate survival (from diabetes) function. Gehan's Wilcoxon's test compared the product limit functions.

RESULTS

The effect of administering a 100×10^3 U dose of rIFN-α (intraperitoneally three times a week for 4 weeks) on the development of diabetes is depicted in Fig. 1. rIFN-α decreased the development of diabetes as assessed by survival curve analysis (Fig. 1) ($P < 0.001$). At 25 weeks of age, no rIFN-α–injected animal (n = 8) developed diabetes as compared with 75% of saline-treated mice (n = 8) ($P < 0.001$). Although treatment ceased at 10 weeks of age, the diabetes-sparing effect was still present at 25 weeks of age when the experiment was terminated. The mean weekly weights of rIFN-α– and saline-injected nondiabetic mice were similar throughout the treatment period (data not shown). rIFN-α–injected mice did not exhibit any abnormal behavior, such as lethargy, diarrhea, hunching, or ruffled hair.

The effect of rIFN-α doses on the development of diabetes was assessed by comparing the effect of 5×10^3 (n = 8), 20×10^3 (n = 8), and 100×10^3 (n = 7) U doses of rIFN-α and saline (n = 10) (administered intraperitoneally three times a week for 4 weeks). The diabetes-sparing effect of rIFN-α administration increased dose dependently (Fig. 2). The 100×10^3 U rIFN-α dose maximally inhibited the development of diabetes ($P < 0.01$) and decreased the final incidence of diabetes to only 14% (a single affected animal) as compared with a 70% diabetes incidence in control animals ($P < 0.01$). The age at diabetes onset in the single rIFN-α–treated mouse developing diabetes was 21 weeks, more than 4 SDs above the mean age at diabetes onset found in control animals (18.4 ± 0.57 weeks). Using a larger group of mice, the 20×10^3 U rIFN-α dose significantly decreased the incidence of diabetes from 72% (13/18) in control animals to 36% (5/14) in treated animals ($P < 0.05$).

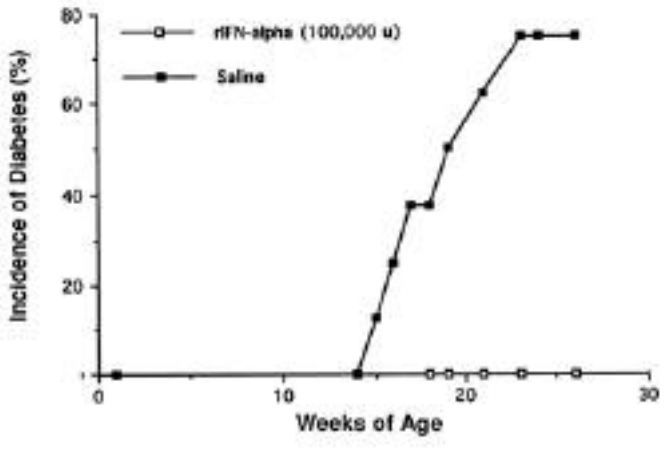

FIG. 1. Incidence of diabetes in female NOD mice administered rIFN-α (100×10^3 U) or saline (n = 7) intraperitoneally three times a week for 4 weeks beginning at 5 weeks of age.

Because 25-week-old mice spared from diabetes by rIFN-α at the termination of the experiment could potentially develop diabetes soon thereafter, a glucose tolerance test was performed on four nondiabetic rIFN-α–treated animals. Glucose tolerance testing of four nondiabetic mice revealed a wide range of serum glucose excursions. Yet, none of the animals were glucose intolerant. Mean (± SE) blood glucoses at 0, 30, 60, and 120 min were 3.99 ± 0.33, 6.98 ± 0.78, 5.18 ± 0.51, and 4.28 ± 0.17 mmol/l, respectively.

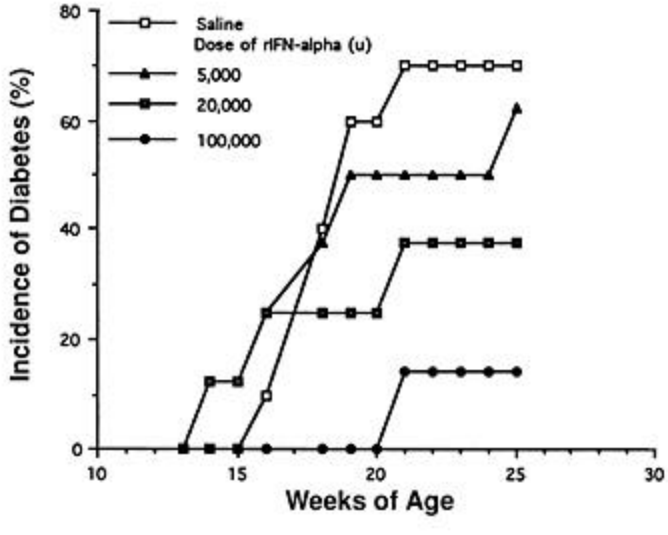

FIG. 2. The dose-dependent effect of rIFN-α on the development of diabetes. rIFN-α at doses of 5×10^3, 20×10^3, and 100×10^3 U was administered intraperitoneally three times a week for 4 weeks.

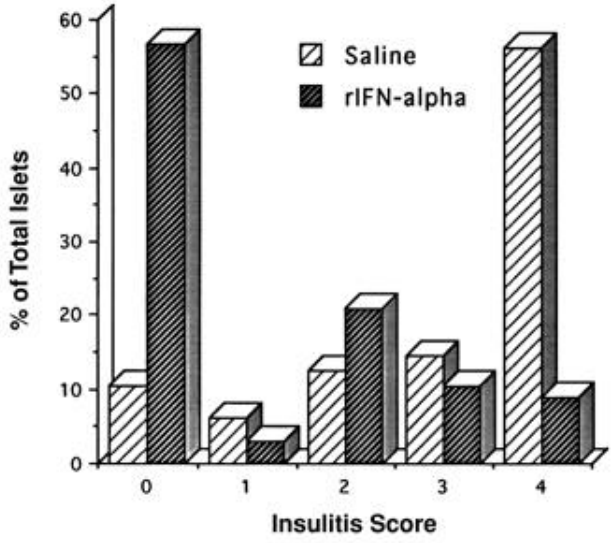

FIG. 3. The frequency of islets from saline- and rIFN-α–treated mice with individual insulitis scores. NOD mice were injected intraperitoneally with saline (n = 5) or rIFN-α (n = 5) (100 × 10^3 U) three times a week. Islets from 8-week-old saline-treated (48 islets) and rIFN-α–treated (67 islets) mice were assigned histopathological scores as described in METHODS.

Histopathological analysis of mouse pancreases (5/group) after 4 weeks of treatment revealed a 65% lower (P < 0.01) mean (± SE) insulitis score in IFN-α–treated mice as compared with control animals (1.19 ± 0.16 vs. 3.16 ± 0.28) (Fig. 3). Islet inflammation consisted of mononuclear leukocytes with few granulocytes. The quality of insulitis process, peri-islet versus intra-islet, was similar in the saline- and rIFN-α–administered mice. The exocrine tissue was spared from the inflammatory response. Islet destruction and atrophy were found mainly in the pancreases of control animals.

The total white blood cell and differential counts after 4 weeks of injections were similar in rIFN-α– (10^5 U dose) (n = 4) and saline- (n = 4) administered mice (7.0 × 10^3 ± 1.1 vs. 6.35 × 10^3 ± 1.2 cells/mm^3, respectively). Mean hematocrits in rIFN-α– and saline-treated mice were also similar (40.1 ± 0.8 vs. 42.3 ± 0.4, respectively).

To further determine whether rIFN-α administration induces a state of generalized immunosuppression, the effect of rIFN-α administration on the induction of a primary immune response to tetanus antigen was assessed. The mean (ΔCPM ± SE) in vitro proliferative responses of mononuclear spleen cells to tetanus antigen were similar in rIFN-α–treated and control mice (21,695 ± 4,485 vs. 23,929 ± 1,270, respectively).

Flow cytometric analysis of mononuclear spleen cells was assessed after 4 weeks of injections. Mice in rIFN-α (n = 4) and control groups (n = 4) had similar proportions of $CD4^+$, $CD8^+$, $B220^+$ (B-cell), and $F4/80^+$ (macrophage) mononuclear spleen cells (Table 1).

rIFN-α administration decreases splenocyte anti-islet effector cell activity. The effect of rIFN-α administration on splenocyte anti-islet effector cell activity was examined by comparing the development of diabetes in irradiated recipient mice receiving splenocytes from rIFN-α and saline-treated animals. The rate of diabetes development was significantly slower in mice administered splenocytes from rIFN-α–treated animals (P < 0.01) (Fig. 4). Also, the cumulative incidence of diabetes was lower in rIFN-α–administered mice (25 vs. 100% in control) (P < 0.02).

TABLE 1
The effect of rIFN-α administration on B-cells, T-cell subsets, and macrophage number

	CD4	CD8	B220	F4/80
Saline-treated mice	38.3 ± 2.1	13.7 ± 0.2	33.6 ± 0.9	4.3 ± 0.3
rIFN-α–treated mice	40.4 ± 3.7	14.2 ± 0.3	35.3 ± 0.2	5.0 ± 0.2

Data are means ± SE.

rIFN-α administration prevents the development of passively transferred diabetes. Pretreatment of NOD mice with rIFN-α potently inhibited the development of passively transferred diabetes after the administration of spleen cells from diabetic mice (P < 0.01) (Fig. 5). The final incidence of diabetes was lower in mice pretreated with rIFN-α (0%) versus saline (90%) (P < 0.01).

In vitro rIFN-α modulates the ability of NOD spleen cells to augment T-cell proliferation. Con A increased NOD and Balb/c syngeneic responder cell proliferation (Fig. 6). This proliferative response was increased when Balb/c or NOD irradiated spleen cells were coincubated with syngeneic responder cells. This augmented proliferative response was dose dependently decreased when NOD irradiated test spleen cells were preincubated with rIFN-α. Preincubation of NOD cells with 20 and 200 U/well rIFN-α decreased Con A proliferative responses to 67 and 54% of control cells (no rIFN-α), respectively (P < 0.05, P < 0.02). Preincubation of Balb/c cells with rIFN-α had no effect on Con A–induced proliferation of responder cells. rIFN-α did not increase vital blue staining of incubated spleen cells (data not shown).

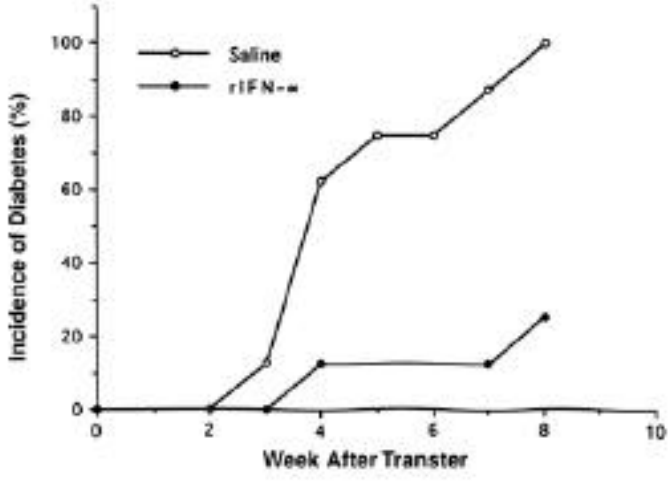

FIG. 4. The effect of rIFN-α administration on anti-islet effector cell activity. Mononuclear splenocytes (4 × 10^7 cells) were isolated from rIFN-α–treated (10^5 U) and saline-treated female NOD mice and were each intravenously administered to two groups (n = 8 per group) of irradiated (750 rads) 10-week-old recipient NOD mice. The rate of diabetes development was slower in recipient mice injected with cells from rIFN-α–treated animals (P < 0.01).

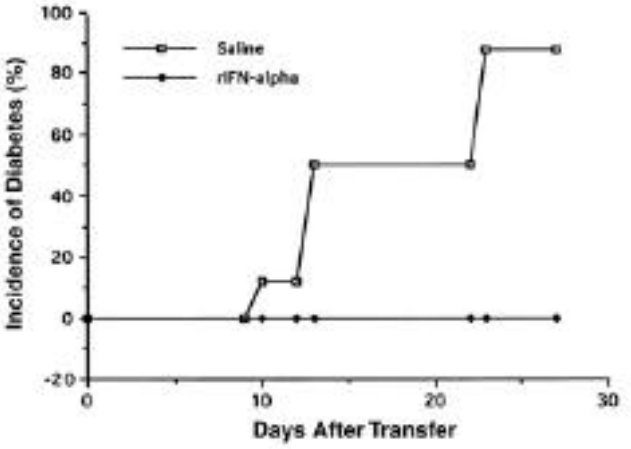

FIG. 5. Effect of rIFN-α administration on the passive transfer of diabetes. Five-week-old male NOD mice were treated with saline (n = 8) or IFN-α (n = 8) (10^5 U) intraperitoneally three times a week for 5 weeks. One week after the cessation of therapy, mice were irradiated (750 rads) and injected intravenously with mononuclear spleen cells (4×10^7 cells/recipient) from diabetic mice. The rates of diabetes development were compared with animals receiving cells from saline-treated and rIFN-α–treated animals.

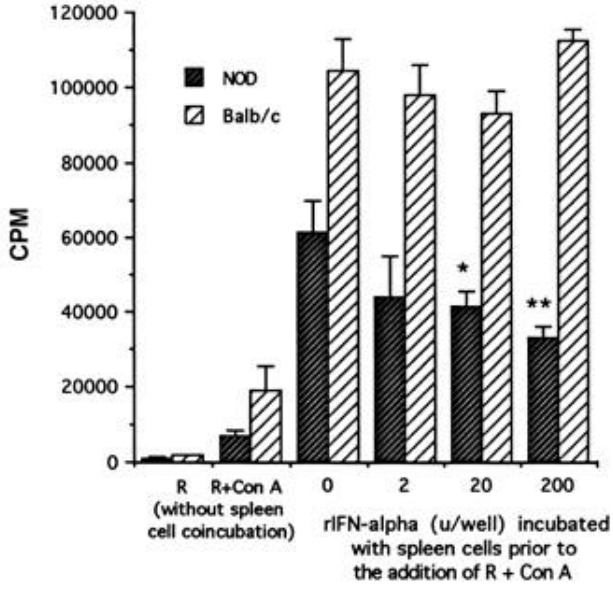

FIG. 6. Effect of in vitro rIFN-α incubation on the modulatory effect of mononuclear spleen cells to augment T-cell proliferation to Con A. Mononuclear spleen cells (10^5 cells/well) from 6-week-old NOD and Balb/c mice were incubated for 2 days with rIFN-α at doses of 0, 2, 20, and 200 U/well. Cells were then washed, irradiated (1,500 rads), and incubated with syngeneic spleen cells (serving as responder cells [R], at a test cell:responder cell ratio of 3:1) and Con A (1 μg/ml). After 72 h, [^{3}H]thymidine (0.5 μCi) was added to each well, and the cells were harvested 16 h later and counted for radioactivity. *P < 0.05 vs. spleen cells preincubated with no rIFN-α and coincubated with R+Con A; **P < 0.02 vs. spleen cells preincubated with no rIFN-α and coincubated with R+Con A.

DISCUSSION

This is the first report demonstrating that rIFN-α administration inhibits the development of diabetes in the NOD mouse. Our data reveal that rIFN-α inhibited diabetes development in a dose-dependent fashion. A dose of 20×10^3 U was effective in decreasing the diabetes incidence by 50%, while a 100×10^3 U dose resulted in maximal inhibition. The latter dose was consistently potent on repeated experiments in that only 0–14% of rIFN-α–treated versus 70–100% of saline-treated mice developed diabetes.

The clinical effectiveness of rIFN-α administration to induce very long-term inhibition of disease was demonstrated by the results of glucose tolerance testing of 25-week-old rIFN-α–treated nondiabetic mice. All these rIFN-α–administered mice exhibited entirely normal glucose tolerances, which suggests that diabetes may be prevented by rIFN-α treatment.

The diabetes-sparing effect of rIFN-α could be mediated by the ability of rIFN-α to protect a target (22), in this case, the β-cell, from immune attack or by inhibiting the immune process. Our data support the later mechanism, since the mean insulitis score of rIFN-α–treated mice was 60% lower than the insulitis score of control mice. To explore the mechanism of how rIFN-α decreases the development of insulitis, the effect of rIFN-α administration on splenocyte anti-islet cytotoxic activity was examined. We found the development of diabetes in irradiated NOD mice was decreased in animals receiving spleen cells from rIFN-α–treated NOD mice as compared with irradiated mice receiving cells from saline-treated mice. These data support the hypothesis that rIFN-α treatment inhibits insulitis and in turn diabetes by depressing anti-islet effector cell activity.

rIFN-α administration potently protected NOD mice from the passive transfer of diabetes with spleen cells (Fig. 5). The potency of this diabetes-sparing effect of rIFN-α is underscored by the findings that the protection was total and the protection was against the infusion of highly activated spleen cells, which quickly causes diabetes in control animals. The protection of these pretreated and irradiated mice taken together with the finding that rIFN-α decreases anti-islet effector cells suggests that rIFN-α decreases anti-islet effector cells through a radioresistant cell(s) and not by directly inhibiting anti-islet effector cells, since effector spleen cells were never exposed to rIFN-α.

Although the induction of a state of generalized immunosuppression can inhibit the development of diabetes, rIFN-α did not appear to induce such a state. Flow cytometric analysis of spleen cells revealed no evidence that alterations of specific T-cell phenotypes or B-cell numbers mediate the diabetes-sparing activity of rIFN-α. White blood cell count was also unaltered. The greatest evidence against an rIFN-α–induced immunosuppression comes from our finding that rIFN-α administration does not decrease the induction of a primary cell–mediated immune response to tetanus antigen.

Immunostimulation of NOD mice with poly I:C administration has recently been demonstrated by Serreze (23) to inhibit diabetes in NOD mice. The findings that poly I:C, a potent inducer of rIFN-α (23), augments macrophage IFN-α mRNA levels in NOD mice while inhibiting the development of diabetes (24), together with our results that rIFN-α inhibits the diabetic process, suggest that the diabetes-sparing effect of poly I:C is mediated by IFN-α.

Serreze (25) has demonstrated defective activation of suppressor cell function in NOD mice, a defect that may be important to the pathogenesis of diabetes and that is corrected with poly I:C administration. Because IFN-α has been

previously shown to induce suppressor cell activity (26,27), rIFN-α administration could be correcting the suppressor cell defect, resulting in the observed decreased islet effector cell activity and prevention of diabetes. Alternatively, rIFN-α could be directly inhibiting the development of anti-islet immune effector cells and diabetes by the property of rIFN-α to decrease the expression of IFN-γ–induced major histocompatibility complex (MHC) class II molecules (28) and in turn decrease pathogenic helper T-cell or cytotoxic T-cell MHC class II–restricted cell-to-cell interactions (29).

In vitro studies demonstrated that Con A–induced responder cell proliferation is augmented when coincubated with either NOD irradiated spleen cells or Balb/c spleen cells. This stimulatory effect is reduced when NOD spleen cells, but not Balb/c spleen cells, are preincubated with rIFN-α before irradiation and coincubation with responder cells and Con A. These data are consistent with the hypothesis that mononuclear cells support a process of T-cell activation that is important in mediating the pathogenesis of diabetes, such as the induction of anti-islet effector cells, and that this support of T-cell activation and the ensuing diabetic process can be inhibited by IFN-α. The decreased anti-islet effector cell activity observed in our transfer study could thus be caused by the rIFN-α–mediated inhibition of (pathogenic) T-cell activation observed in vitro. Further, these in vitro data, taken together with the observation that IFN-α administration of recipient NOD mouse protects against the adoptive transfer of diabetes, suggest that the adoptive transfer of diabetes with mononuclear splenocytes requires a radioresistant cell in the recipient that supports T-cell function or activation, and that this cell activity can be inhibited by IFN-α treatment of the recipient animal. The in vitro action of rIFN-α does not appear to represent the in vitro induction of suppressor-like cells because preincubation of NOD cells with rIFN-α did not reduce proliferative responses to levels (CPM) lower than that found when responder cells are incubated with Con A alone without the coincubation of irradiated NOD spleen cells. However, these in vitro data do not rule out the possibility that (radioresistant) immunoregulatory cells are induced by IFN-α in vivo and in turn decrease anti-islet effector activity and diabetes development.

In vitro rIFN-α did not alter the stimulatory effect of Balb/c spleen cells on T-cell proliferation. It is unclear why NOD spleen cells are sensitive to rIFN-α and Balb/c spleen cells are not. However, in vitro sensitivity of mononuclear cells from subjects with another T-cell–mediated autoimmune disorder to a type I interferon has been previously reported. Noronha (30) demonstrated that peripheral blood cells from subjects with multiple sclerosis were more sensitive to the in vitro induction suppressor cells by IFN-β than cells from normal control subjects.

The role of IFN-α in the pathogenesis of disease has been suggested with the demonstration that transgenic mice expressing IFN-α within islets develop diabetes (13). IFN-α mRNA has also been found increased in islets of streptozotocin-induced diabetic mice (14) and DP BB rats (14) and in the pancreases of people with IDDM (16). Further, elevated blood levels of IFN-α have been found in serum of subjects with autoimmune disorders, including IDDM (31). However, the cause-and-effect relationship of increased levels of immunoreactive IFN-α with diabetes has not been shown. The paradox of why transgenic mice expressing IFN-α in islets develop diabetes while rIFN-α administration prevents diabetes in NOD mice may be explained by several possible reasons. The different tissue levels of IFN-α present in the transgenic mice and rIFN-α–administered NOD mice could result in opposite biologic effects. Indeed, the administration of very different doses of another cytokine, IL-1, has opposite effects on the development of diabetes in the BB rat (32). The different time(s) during the course of the disease that IFN-α levels are elevated in the transgenic mice and in rIFN-α–treated animals presented herein may result in different immunological effects, since IFN-α can either augment or suppress cellular and humoral immunity depending upon the time during the course of disease that rIFN-α is administered (33). Interestingly, different frequencies of administration of another cytokine, IL-12, can either prevent or accelerate diabetes in NOD mice (34,35). Although the cause is unknown, the phenomenon that a cytokine can inhibit the diabetic process when injected, yet induce diabetes when expressed in islets of transgenic mice, has been previously described (36,37).

The diabetes inhibitory effect of rIFN-α administration may appear surprising in light of our previous demonstration that treatment of BB rats with poly I:C, an inducer of IFN-α, accelerates the development of diabetes (38). This paradox may be explained by the use of different animal models. One difference includes the presence of islet IFN-α mRNA in BB rats but not in NOD mice (14). However, other reasons exist. First, as mentioned above, different tissue levels of IFN-α present in poly I:C– and rIFN-α–treated animals or the different times during the course of disease that IFN-α is augmented may lead to dichotomous biologic results (32,33). Alternatively, poly I:C treatment in rats could accelerate diabetes by its ability to augment other cytokines, particularly IFN-γ (39) or IL-1 (40), which have been found to be cytotoxic to islets.

The demonstration that rIFN-α prevents diabetes is consistent with the hypothesis that IFN-α mediates the diabetes-sparing effect of LCMV, an inducer of IFN-α. Both LCMV infection and rIFN-α administration potently inhibit the development of insulitis and diabetes. Further, live virus, which is required for rIFN-α production, is also needed to inhibit the diabetes process (41).

In conclusion, rIFN-α administration potently prevents diabetes in the NOD mouse by inhibiting the developing of insulitis. This inhibition of the islet inflammatory process appears to be due to an indirect decrease in anti-islet effector cell activity by rIFN-α. This rIFN-α action may have significant implications to further our understanding of the diabetic process and develop a means to inhibit it.

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Author Queries (please see Q in margin and underlined text)

Hard copy did not match diskette version in some places. Please check proofs carefully.

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Q1: Please provide the name of the town on the Cape where the CCA is. Also, Assoc. for associates or association?
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Q2: Closing parenthesis correctly placed?
Q3: OK to add “in treated animals” for clarity?
Q4: Noronha in References vs. Nornha in text.
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Q6: Last two sentences seem to be saying the same thing.