Original Articles

Poly I:C Induction of α-Interferon in the Diabetes-Prone BB and Normal Wistar Rats

Dose-Response Relationships

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Although the administration of a fixed dose of the α-interferon (α-IFN) inducer, polyinosinic polycytidilic acid (poly I:C), accelerates the development of diabetes in DP-BB rats, no reports have characterized the dose-response relationship of poly I:C with serum α-IFN levels and the development of diabetes. This study examines the dose-response relationships of poly I:C with the induction of serum α-IFN and the development of diabetes in DP-BB and normal Wistar rats. Also tested in this study is the hypothesis that the lack of development of diabetes in poly I:C-treated normal Wistar rats is attributable to a deficient α-IFN response. Using poly I:C doses of 0.5, 1.5, 5, and 10 μg/g body weight, a direct dose-response relationship was observed in DP-BB rats with the serum α-IFN response. Moreover, all doses of poly I:C accelerated the onset of diabetes in BB rats. Serum α-IFN levels inversely correlated with time of onset of diabetes ($P < 0.01$). Also, BB rats with higher levels of serum α-IFN were associated with earlier onset of diabetes ($P < 0.001$). Poly I:C-induced serum α-IFN levels were significantly lower in diabetic than in nondiabetic BB rats. In normal Wistar rats, although all doses of poly I:C significantly increased serum α-IFN levels, diabetes was not induced. The results of this study indicate that poly I:C administration elevates serum α-IFN and accelerates the development of diabetes in BB rats at even very low doses. This finding in conjunction with the correlation of serum α-IFN with the onset of diabetes is consistent with a pathogenetic role of α-IFN. The absence of a diabetogenic effect of poly I:C in Wistar rats suggests that α-IFN alone cannot induce diabetes in this animal. *Diabetes* 43:518–22, 1994

Type I diabetes mellitus is an autoimmune disorder in which cytokines have been speculated to play a pathogenetic role (1–4). Based on several observations, α-interferon (α-IFN) has been speculated previously to play a role in the development of autoimmune disorders, including diabetes (5). Serum and synovial fluid α-IFN levels and levels of 2′-5′ oligoadenylate synthetase, an enzyme induced by α-interferon, are elevated in subjects with autoimmune disorders (6–9). In addition, α-IFN has been detected in the pancreatic islets of humans with newly onset type I diabetes and has been demonstrated to inhibit insulin release (10,11).

The administration of polyinosinic polycytidilic acid (poly I:C) has been shown to accelerate the development of diabetes in DP-BB rats (12,13), a model of human diabetes (14,15), and in DR-BB rats (13,16). Because poly I:C induces the release of cytokines (17–19), particularly α-interferon, these substances have been speculated to play a major role in the pathogenesis of diabetes. Of these cytokines, we have found serum α-IFN levels elevated in BB rats after the injection of a fixed dose (5 μg/g body weight) of poly I:C (12). However, no reports characterize the dose-response relationships of poly I:C with serum α-IFN levels and the development of diabetes.

We have shown previously that normal Wistar rats treated with the same fixed dose of poly I:C do not develop diabetes (12). Yet the effect of administering higher doses of poly I:C on the development of diabetes and the induction of serum α-IFN has not been reported. In addition, the hypothesis that the lack of development of diabetes in poly I:C-treated Wistar rats is attributable to a deficient α-IFN response has not been tested.

This study examines the dose-response relationships of poly I:C on the induction of serum α-IFN over time and on the development of diabetes in both DP-BB rats and normal Wistar rats. In addition, because other immune responses are blunted in humans with diabetes (20), this study also examines the effect of the diabetic state on the capacity of poly I:C to induce α-IFN.

RESEARCH DESIGN AND METHODS

DP-BB rats were purchased from the University of Massachusetts Medical School (Worcester, MA). To establish a viral pathogen-free environment, the drinking water was acidified and the bedding and cages, which were autoclaved before use, covered with filter bonnets, and placed in laminar flow hoods. Wistar rats, the parental strain of the BB rat, known not to be genetically susceptible to diabetes, were purchased from the Charles River Company (Wilmington, MA). All rats received food and water ad libitum. Poly I:C (Sigma, St. Louis, MO) was diluted in saline to a concentration of 10 mg/ml and stored at −40°C.

Experiments were performed on DP-BB rats that were divided into the following four treatment groups and a control group: 0.15 M sodium

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α-IFN, α-interferon; poly I:C, polyinosinic polycytidilic acid; IL, interleukin.

chloride (saline) (n = 20); poly I:C at 0.5 μg/g body weight (poly-0.5) (n = 10); poly I:C at 1.5 μg/g (poly-1.5) (n = 5); poly I:C at 5 μg/g (poly-5) (n = 5); and poly I:C at 10 μg/g (poly-10) (n = 5). From 40 to 47 days of age, saline or the designated dose of poly I:C was administered intraperitoneally three times a week (three times per week) for 6 weeks or until the day of diabetes diagnosis. Blood glucose concentrations were measured three times per week. Rats were defined as diabetic when blood glucose concentrations exceeded 11.1 mM on two successive days. Rats were sacrificed at the time of diagnosis of diabetes or at 130 days in the case of nondiabetic animals. Serum α-IFN was measured before (0 time), 6 h (α-IFN at 6 h), and 24 h (α-IFN at 24 h) after the first poly I:C injection of each week. The net increase of serum α-IFN levels from baseline to 6 h (α-IFN at 0–6 h) and to 24 h (α-IFN at 0–24 h) were also determined and compared.

The effect of the diabetic state on the ability of poly I:C to augment serum α-IFN was assessed by comparing the serum α-IFN at 6 h and α-IFN at 0- to 6-h values in 9 poly I:C-induced diabetic and 9 aged-matched nondiabetic DP-BB rats that were administered poly I:C three times per week since 47 days of age. In addition, the effect of poly I:C on BB rats spontaneously developing diabetes was examined. In 5 previously poly I:C-untreated newly diabetic rats, serum α-IFN at 6 h and α-IFN at 0- to 6-h levels were measured after only a single dose of poly-5.

The effect of poly I:C on serum α-IFN levels and the development of diabetes was determined in normal Wistar rats. Rats were divided into four treatment groups. From 52 to 82 days of age, Wistar rats were administered either saline (n = 4), poly 1.5 (n = 3), poly 5 (n = 6), or poly-10 (n = 3), intraperitoneally three times per week. Serum α-IFN levels 6 h after the first injection of weeks 2 and 3 were compared in each treatment group.

Analysis of serum α-IFN. Serum α-IFN levels were assayed using a biological method that determines the inhibition of cytopathic effects of vesicular stomatitis virus (American Type Culture Collection, Rockville, MD) on rat kidney cells (Lee Biomolecular, San Diego, CA) (21). Neutralization of test sera with α-IFN antisera was used to assess specificity of α-IFN activity. Rat α-IFN standard and antiserum were obtained from Lee Biomolecular. The results are expressed as units of α-IFN, which is the reciprocal of the titer of serum that showed a 50% inhibition of cytopathic effect.

Statistical analysis. Group comparisons were analyzed by analysis of variance. Differences between paired samples were compared with a paired Student's t test. The product-limit method of Kaplan and Meier stimated survival (from diabetes) function, which was compared with the Gehan's Wilcoxon test.

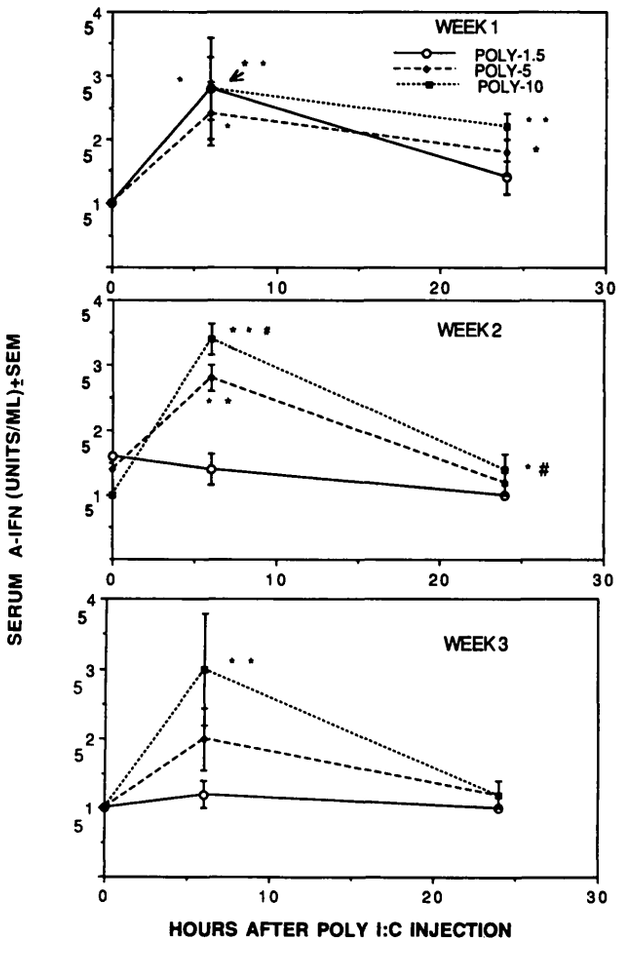

FIG. 1. Serum levels of α-IFN (A-IFN) at 0, 6, and 24 h after the administration of poly-1.5, poly-5, and poly-10 to DP-BB rats. *P < 0.05 vs. 0 time for same poly I:C dose. **P < 0.01 vs. 0 time for same poly I:C dose. #P < 0.05 vs. poly-1.5 dose at the same point.

RESULTS

Figure 1 shows the dose-response relationship of poly I:C (poly-1.5, poly-5.0, and poly-10) with serum α-IFN at 6- and 24-h levels at weeks 1, 2, and 3. An overall dose-response relationship occurred between the dose of poly I:C and serum α-IFN levels. Poly-0.5 did not augment serum α-IFN levels at any week (data not shown). Serum α-IFN at 6-h levels of poly-5– and poly-10–treated rats were significantly greater than the serum α-IFN at 6-h levels in saline-treated rats at weeks 2 (1.08 ± 0.08) and 3 (1.0 ± 0.0) (data not shown).

At week 1, poly I:C-1.5, poly-5.0, and poly-10 significantly increased serum α-IFN after 6 h over baseline levels (Fig. 1), whereas only poly-5 and poly-10 increased serum α-IFN at 6 h at weeks 2 and 3. Serum α-IFN at 24-h levels were increased over baseline levels by the higher doses at week 1 and by poly-10 at week 2.

The net increases of serum α-IFN at 6 h from baseline values (α-IFN at 0–6 h) and of serum α-IFN at 24 h from baseline (α-IFN at 0–24 h) are depicted in Fig. 2. Serum α-IFN at 0–6 h increased dose (poly I:C) dependently at weeks 2 and 3. Serum α-IFN at 0–24 h rose dose dependently at weeks 1 and 2.

The relationship of the average serum α-IFN at 6-h levels induced by poly-1.5, poly-5.0, and poly-10 over weeks 1, 2, and 3 of each poly I:C-treated and control rat with the administered poly I:C dose was assessed. A direct correlation exists between the dose of poly I:C and the average serum α-IFN at 6-h levels of weeks 1, 2, and 3 (r = 0.77) (P < 0.001) (Fig. 3). Because poly-0.5 did not increase serum

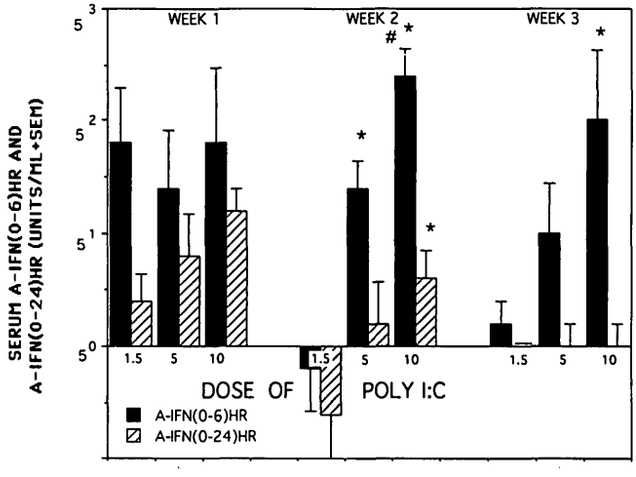

FIG. 2. Serum α-IFN-(0–6) h and α-IFN-(0–24) h levels (A-IFN) at 1, 2, and 3 weeks after the administration of poly-1.5, poly-5, and poly-10 to DP-BB rats. Comparisons are made between α-IFN at 0- to 6-h levels and between α-IFN at 0- to 24-h levels. *P < 0.5 vs. poly-1.5 at same week. #P < 0.05 vs. poly-5.0 at same week.

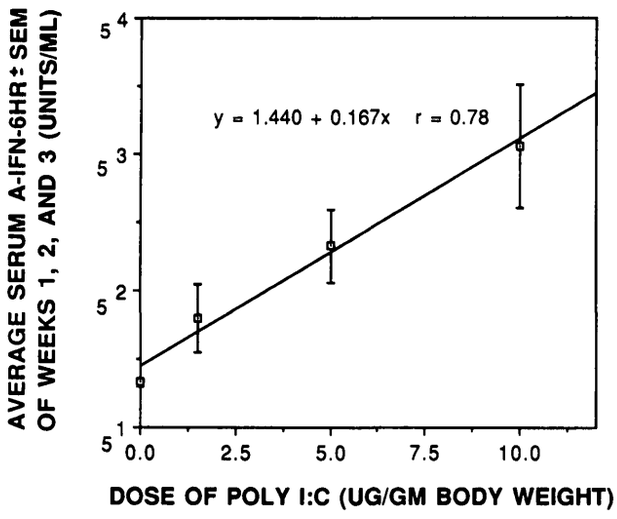

FIG. 3. The correlation of the mean serum α-IFN-6 h (A-IFN) levels at weeks 1, 2, and 3 with the dose of poly I:C (0, 1.5, 5.0, and 10.0 μg/g body weight) administered to DP-BB rats ($P < 0.001$).

α-IFN levels, these results have been omitted.

The cumulative incidence of diabetes of all treatment groups is shown in Fig. 4. By comparing survival analysis curves, all doses of poly I:C significantly accelerated the development of diabetes over saline controls by 70 days of age ($P < 0.005$). By 130 days of age, all doses, save the lowest dose (poly-0.5), accelerated the development of diabetes over control rats ($P < 0.002$ for poly-1.5, $P < 0.001$ for poly-5,

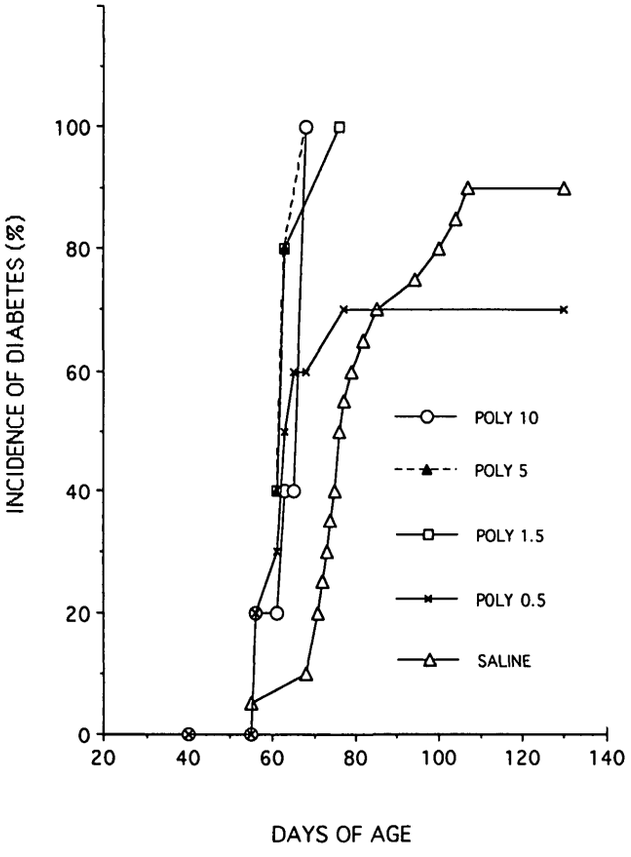

FIG. 4. The incidence of diabetes after the administration of poly-0.5, poly-1.5, poly-5.0, and poly-10 intraperitoneally three times per week to DP-BB rats.

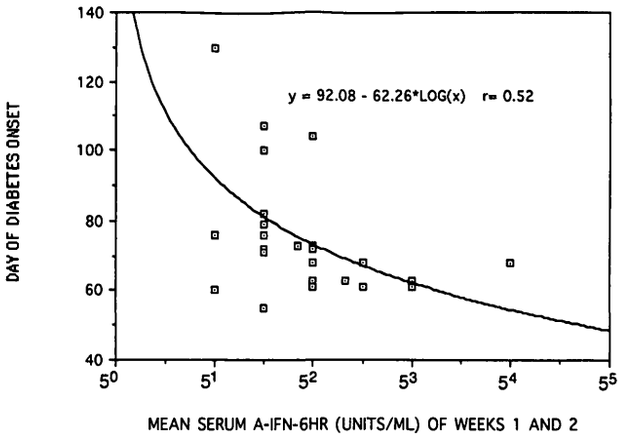

FIG. 5. The correlation between the mean serum α-IFN at 6-h levels (A-IFN) of weeks 1 and 2 and the day of onset of diabetes in DP-BB rats administered saline ($n = 13$), poly-0.5 ($n = 5$), poly-1.5 ($n = 5$), poly-5.0 ($n = 5$), and poly-10 ($n = 5$), intraperitoneally three times per week.

and $P < 0.001$ for poly-10). Although 100% of the rats treated with the higher poly I:C doses (poly-1.5, poly-5, and poly-10) became diabetic, only 70% of rats treated with poly-0.5 became diabetic.

The mean (± SE) age of diabetes onset in diabetic rats treated with poly-0.5, poly-1.5, poly-5.0, and poly-10 were similar (63.0 ± 2.8, 64.8 ± 2.8, 63.2 ± 1.2, and 65.6 ± 1.5 days) and individually and collectively significantly less than the mean age of diabetes onset in saline controls (84.9 ± 4.4 days, $P < 0.03$).

The development of diabetes in BB rats in all treatment groups was related to serum α-IFN. The average serum α-IFN at 6-h levels ($n = 33$) of weeks 1 and 2 correlated with the day of onset of diabetes ($P < 0.01$) (Fig. 5). Further, animals with higher mean serum α-IFN at 6-h levels at weeks 1 and 2 were associated with earlier (before 75 days) onset of diabetes ($\chi^2 = 10.9$, $P < 0.001$).

The effect of the diabetic state on the ability of poly I:C to induce α-IFN was assessed in poly I:C-induced and spontaneously developed diabetes. Serum α-IFN at 6-h levels were compared in aged-matched (61–63 days of age) newly diabetic ($n = 9$) and nondiabetic ($n = 9$) BB rats administered poly I:C since 47 days of age (Fig. 6). The poly I:C-treated diabetic rats had lower mean serum α-IFN levels at 6 h than poly I:C-treated nondiabetic rats ($P < 0.01$). Serum α-IFN at 6-h levels after a single dose of poly-5 was lower in BB rats ($n = 5$) spontaneously developing newly onset diabetes than in nondiabetic rats ($P < 0.05$). Poly-5 did not significantly augment serum α-IFN above baseline values in BB rats with either poly I:C-induced or spontaneously developed diabetes.

In normal Wistar rats, serum α-IFN at 6-h levels after the administration of saline and poly I:C were compared after the first injection of weeks 2 and 3 (Fig. 7). Serum α-IFN at 6-h levels were greater in all poly I:C-treated groups than in saline-treated control rats at both weeks 2 and 3. Using paired analysis of α-IFN response to poly I:C at weeks 2 and 3, serum α-IFN at 6-h levels were significantly lower at week 3 than at week 2 ($P < 0.04$). Serum α-IFN levels in saline-treated rats were not altered over time. No poly I:C-treated Wistar rat developed diabetes nor a significant degree of insulitis. Only an occasional inflammatory cell was found in the islets.

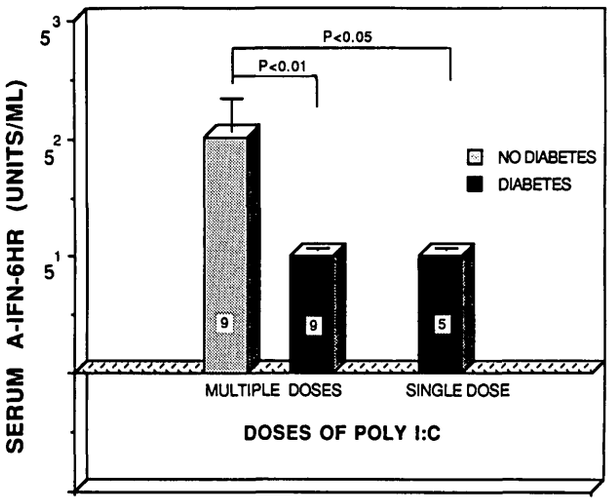

FIG. 6. Serum α-IFN at 6 h (A-IFN) in 61- to 63-day-old diabetic and nondiabetic DP-BB rats receiving poly-5 three times per week since 47 days of age and serum α-IFN at 6 h after a single dose of poly-5 to DP rats that developed diabetes spontaneously.

DISCUSSION

We and others (12,13) have demonstrated previously that the administration of poly-5 accelerates the development of diabetes in DP-BB rats but does not induce diabetes in the Wistar rat (12). Further, we (12) have shown previously that serum α-IFN is induced by poly-5 in DP-BB rats. Using a range of poly I:C doses, this study extends these initial observations and examines the poly I:C dose-response relationships with serum α-IFN and the diabetic state in both the DP-BB and Wistar rats.

The data in this study demonstrate that the intraperitoneal administration of poly I:C at doses of 1.5–10 μg/g body weight increases serum α-IFN at 6 h in DP-BB rats. The induction of α-IFN appears to be dose-related because, in many cases, higher doses of poly I:C induce higher serum α-IFN at 6-h, α-IFN at 24-h, and α-IFN at 0- to 6-h levels after the first week of treatment. Additionally, the average serum α-IFN at 6-h levels at weeks 1, 2, and 3 for each poly I:C dose correlates positively with the administered dose of poly I:C.

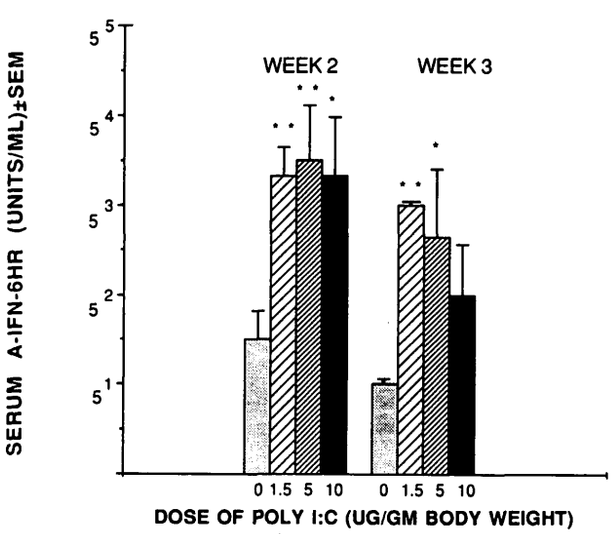

FIG. 7. Serum α-IFN-6 h (A-IFN) levels at 2 and 3 weeks after the administration of saline, poly-1.5, poly-5.0, and poly-10 to normal Wistar rats. *$P < 0.05$ vs. saline **$P < 0.01$ vs. saline.

All doses of poly I:C, even as low as 0.5 μg/g body weight, accelerate the development of diabetes by 70 days. Also, a relationship appeared between the poly I:C dose and the development of diabetes. By 130 days, only rats treated with the lowest dose of poly I:C (0.5) had an overall development of diabetes no different from saline-treated rats. Also, only 70% of the rats treated with the lowest dose (poly-0.5) developed diabetes, as compared with 100% of the rats treated with the higher doses (poly-1.5, poly-5, and poly-10).

An important relationship was found between serum α-IFN levels and the development of diabetes. Not only were serum α-IFN levels associated with early development of diabetes, but serum α-IFN at 6-h levels were inversely correlated with the age of onset of diabetes. In addition, the lowest poly I:C dose did not increase serum α-IFN levels, as did the higher doses, and induced the lowest incidence of diabetes by 130 days. Taken together, this data is consistent with, but does not definitely prove, a pathogenetic role of α-IFN in the development of diabetes in the BB-DP rat.

Further support for the role of α-IFN in the pathogenesis of diabetes has been provided with the recent demonstration that transgenic mice, which express α-IFN in β-cells, develop insulitis and diabetes (22). Moreover, the degree of insulitis was lowered with anti-α-IFN administration. Although poly I:C classically augments α-IFN, it may also increase cytokines cytotoxic to islets, such as γ-IFN, interleukin (IL)-1, and IL-2, (3,4,23) which in turn may alternatively play a role mediating the effect of poly I:C.

The results of this study demonstrate that the diabetic state that either develops spontaneously or is induced by poly I:C impedes the ability of the DP-BB rat to respond to poly I:C administration with augmented serum α-IFN levels. The mechanism(s) of this inhibition is not addressed in this study but may be secondary to decreased α-IFN secretion and is consistent with the findings that individuals with diabetes, particularly those in poor metabolic control, have other types of immune deficiencies (20,24,25). The α-IFN response to poly I:C has not been studied in diabetic patients. The depressed serum α-IFN levels may alternatively be attributable to increased α-IFN clearance secondary to increased metabolic breakdown.

In normal Wistar rats, this study shows that poly I:C increases serum α-IFN at 6 h to levels equal to or greater than that found in poly I:C-treated BB rats. In fact, only in the Wistar rats was the mean α-IFN at 6-h levels greater in poly-1.5 than in the saline group at both weeks 2 and 3. In Wistar rats, α-IFN induction by poly I:C is significantly reduced from weeks 2 to 3. This relative hyporesponsive state to repeated doses of poly I:C has been demonstrated previously in mice (26,27) and humans (28), but not in rats.

We have reported previously that the administration of a fixed dose of poly I:C (poly-5) is ineffective in inducing diabetes in normal Wistar rats (12). This study demonstrates that even with a higher poly I:C dose (poly-10), none of the poly I:C-treated Wistar animals develop diabetes or insulitis. Furthermore, our data show that the inability of poly I:C to induce diabetes in Wistar rats cannot be attributed to a deficient α-IFN response to poly I:C. Perhaps some other genetic component(s) providing immunological susceptibility to the development of diabetes are necessary factors for this poly I:C action. Alternatively, there may be cytokines or immune modulators other than α-IFN that are augmented by poly I:C in DP-BB rats and not in Wistar rats. Future studies

will be required to definitively establish the specific mechanism(s) of poly I:C-accelerated diabetes.

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