Low Dose Poly I:C Prevents Diabetes in the Diabetes Prone BB Rat

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Poly I:C, an inducer of IFN-α and other cytokines, has been used to study the development of diabetes in both the BioBreeding (BB) diabetes prone rat and non-obese diabetic (NOD) mouse animal models of insulin-dependent diabetes mellitus (IDDM). Surprisingly, poly I:C accelerates the disease in the BB rat while inhibiting it in the NOD mouse. Since cytokines can have dose related opposing effects on immune responses, we hypothesized that the paradoxical effect of polyinosinic polycytidylic acid (poly I:C) on diabetes in the two animal models is dose related. Accordingly, we compared the incidence of diabetes and degree of insulitis in diabetes prone BB rats administered saline and poly I:C at doses (0.05 μg/g body weight and 0.1 μg/g body weight) up to 100-fold lower than doses (poly-5 μg/g) previously found to accelerate diabetes. In addition, the non-specific suppressor activity of mononuclear splenocytes from BB rats administered low dose (poly-0.05 μg/g body weight), high dose (poly-5 μg/g body weight), and saline were compared. The development of diabetes was inhibited in rats treated with each dose of poly I:C. The degree of insulitis in poly-I:C treated animals was also less severe. The total white blood cell count and proportion of RT6+ T-cells and each T-cell subset were unaltered by poly I:C. When compared to splenocytes of control animals, splenocytes from poly I:C (0.05 μg/g body weight) treated rats suppressed responder cell proliferation to concanavalin A and alloantigen. However, spleen cells from high dose poly-I:C did not suppress responder cell proliferation to alloantigen. In adoptive transfer studies, the administration of spleen cells from poly-0.05 treated rats decreased the development of diabetes in recipient BB rats. In vitro studies also demonstrated that poly-I:C inhibits the proliferative response of BB rat spleen cells to concanavalin A. The administration of poly-0.05, but not poly-5.0, decreased TNF-α mRNA and IL-10 mRNA content in spleen cells. We conclude that poly I:C, at a dose 100 times lower than that required to accelerate diabetes prevents the development of diabetes in BB rats by interfering with the development of insulitis. The induction of suppressor cell activity induced by low dose poly-I:C in vivo and the inhibition of T-cell responses by poly-I:C in vitro suggests that the diabetes sparing activity of poly I:C is mediated by augmented immunoregulatory cell activity. Further studies with poly I:C may be important in increasing our understanding of the pathogenesis of IDDM and provide a means to prevent it.

Introduction

BioBreeding (BB) diabetes prone rats have been extensively utilized as an animal model for human insulin dependent diabetes mellitus (IDDM), since these rats spontaneously develop diabetes following a cell-mediated autoimmune destruction of beta cells. As in human IDDM, the pathogenesis of disease in the diabetes prone BB rat is via an autoimmune process characterized by lymphocytic infiltration of the islets [1] and by the presence of serum anti-islet cytotoxic antibodies [2]. Unlike human IDDM, lymphopenia [3] and immune deficiencies [4, 5] are found in the BB rat. In both the BB rat and NOD (non-obese diabetic) mouse, another well studied animal model of spontaneous diabetes that shares many immunologic and
pathologic features of human IDDM, depressed regulatory T-cell activity is hypothesized to play an important pathogenetic role [3, 6]. Polyinosinic polycytidylic acid (poly I:C), an inducer of cytokines, increases suppressor T-cell activity and inhibits the development of diabetes in! the NOD mouse [7]. Surprisingly, poly-I:C administration was recently demonstrated to accelerate the development of diabetes in diabetes-prone BB rats and even induce diabetes in diabetes-resistant BB rat strains [8–10]. These contrary effects of poly I:C administration in the different animal models of IDDM are not understood. Since the action of poly I:C is thought to be mediated by the induction of cytokines and since the administration of different cytokine dosages may have opposite effects on the same immune function [11], we hypothesized that poly I:C induces both dose-related inhibitory and excitatory effects on the diabetic process which is poly-I:C dose related. Further, we hypothesized that an inhibitory effect on the diabetic process is mediated by the induction of regulatory cell activity. This study tested these hypotheses by determining if poly I:C, at dosages lower than that required to accelerate diabetes in the BB rat will in vivo prevent the development of insulitis and diabetes and induce non-antigen specific suppressor spleen cells in diabetes prone BB rats. In addition, we determined if poly I:C will in vitro inhibit the T-cell proliferative response to concanavalin A (Con A).

Materials and Methods

Animals

Diabetes prone BB rats were obtained from the viral antibody-free (VAF) colony at the University of Massachusetts (MA, USA). To preserve VAF conditions, animals were housed in sterilized cages which were covered with filter bonnets and placed in a laminar flow hood. Bedding and food were autoclaved and the drinking water was acidified. Principles of laboratory animal care were followed in compliance with Public Health Services Policy as outlined in the ‘Guide for The Care and Use of Laboratory Animals in Research and Teaching’ (PHS 1985 USA).

Poly I:C

Poly I:C (Sigma Chemicals, St. Louis, MO, USA) was resuspended in phosphate buffered saline (PBS) at a stock concentration of 10 mg/ml, aliquoted, and stored frozen (−40°C) prior to use.

Experimental design

Male diabetes-prone BB rats were randomly placed into the following treatment groups: saline (n=22), poly I:C at 0.05 µg/g body weight (poly-0.05) (n=11), and poly I:C at 0.1 µg/g body weight), (poly-0.1) (n=16). Starting at approximately 40 days of age, the respective treatments were administered by intraperitoneal (i.p.) injection, 3 times per week (TIW) for 5 weeks. Blood glucoses were determined TIW and randomly selected rats were weighed weekly. After 3 weeks of treatment, total white blood cell count and differential count were determined. When blood glucoses reached >14 mM on two consecutive days, the animals were diagnosed with diabetes and killed soon after. All diabetic animals exhibited weight loss. Non-diabetic animals were killed at 130 days. However, four non-diabetic poly-I:C treated rats were observed for an additional 4 months.

To compare the effect of low dose (poly-0.05) and high dose poly I:C (poly-I:C 5 µg/gm body weight, poly-5.0) on the induction of suppressor-cell activity, either saline, poly-0.05 (i.p. TIW for 12 doses) or poly-5.0 (i.p. TIW for seven doses) were administered to BB rats. Since diabetes alters immune responses, animals were killed and studied at a time prior to diabetes onset. Since most BB rats injected with poly-5.0 develop diabetes within three weeks of treatment [10], these treated animals were sacrificed and studied after seven poly I:C doses.

Pancreatic histology

Pancreata were fixed in buffered formalin and embedded in paraffin. The tissue was then, sectioned, stained with hematoxylin and eosin, and assessed for islet inflammation under light microscopy in a blinded test. The degree of insulitis was scored by a blinded observer as follows: 0 if no inflammation, 1+ if 1–10% of the islet was infiltrated, 2+ if 10–25%, 3+ if 25–75%, and 4+ for >75% islet involvement or islet fibrosis.

Analysis of cell surface phenotypes of peripheral blood mononuclear cells

Blood was collected by intracardiac puncture and peripheral mononuclear cells (PBMC) were then isolated using Ficoll-Hypaque centrifugation. A minimum of 10,000 cells were analysed by flow cytometry (FacScan, Becton Dickinson, Mountain View, CA, USA). OX19 (pan T-cell phenotype) FITC-conjugated antibody and OX8 (cytotoxic T-cell and natural killer (NK) cell) PE-conjugated antibody (Serotec, Oxford, UK) were utilized to delineate the PBMC phenotypes. OX19+, OX8+ cells were identified as cytotoxic-suppressor T-cells, OX19+, OX8− cells as helper-inducer cells, and OX19−, OX8+ cells as NK cells. RT6.1+ T-cells, reported to have suppressor-like activity, were measured using DS4.23 (a rat MoAb from D. Lubaroff) [12] and goat anti-rat IgG conjugated to PE (Serotec). Class I MHC expression on mononuclear cells was determined utilizing a mouse anti-rat class I MHC antibody (OX18) (Serotec) and goat anti-mouse IgG conjugated to fluorescein isothiocyanate (FITC) (Gibco BRL).
Splenocyte cell suppressor assays

The ability of diabetes-prone BB rat splenocytes to suppress T-cell proliferative responses to alloantigen and Con A was assessed in separate experiments [13]. Diabetes-resistant BB-rat mononuclear splenocytes served as responder cells and were incubated with either irradiated (3,000 rad) allogeneic mononuclear splenocytes from Fisher rats at a responder:allogeneic cell ratio of 1:1 or with Con A (2 μg/ml) in RPMI 1640 media containing 10% fetal calf sera (FCS), 10 mM HEPES, 1 mM sodium pyruvate and 2 mM glutamine. Splenocytes from poly-I:C treated and control BB rats were irradiated (1,000 rads) and individually added to wells with responder cells, in quadruplicate, to achieve suppressor:responder cell ratios ranging from 0.125:1–1:1. After 3 days of incubation, 1 μCi of 3H-thymidine was added to each well. Sixteen hours later, the cells were harvested and counted for radioactivity.

Adoptive transfer experiments to assess immunoregulatory cell activity

The hypothesis that the inhibitory action of poly-0.05 on the diabetic process is mediated by the induction of immunoregulatory cells was studied to determine whether the administration of spleen cells from poly-0.05 treated BB rats inhibits the development of diabetes in recipient BB rats. Mononuclear spleen cells (60×10⁶ cells) from BB rats previously administered either saline or poly-0.05 (i.p. three times for 4 weeks) were injected IV and IP into BB rats at 25 days of age and again at 40 days of age. The development of diabetes was then compared in recipient animals (saline=9, poly-0.05 n=10).

The in vitro effect of poly I:C on mononuclear spleen cells

Mononuclear spleen cells (10⁵ cells/well) from untreated diabetes-prone BB rats were incubated with poly I:C at concentrations of 0, 0.05 and 0.5 μg/ml in RPMI 1640 containing 10% FCS, 10 mM HEPES, 1 mM sodium pyruvate, 2 mM glutamine, 2×10⁻⁵ M 2-mercaptoethanol, and 100 μg/ml Pen/Strep. The cells were washed and incubated with Con A (2 μg/ml) for 48 h. Cells incubated in complete media served as a control for Con A stimulated cells. After 48 h, 3H-thymidine (1 μCi) was added to each well and the cells were harvested 16 h later, and counted for radioactivity. In vitro poly-I:C concentrations were chosen to approximate the blood poly-I:C concentrations that would be expected: (0.05 μg/ml), assuming i.p. injected poly I:C is evenly distributed throughout the body. The highest in vitro concentration approximates a blood poly I:C concentration that assumes poly I:C at doses of 0.05 μg/gm is preferentially distributed ten times higher intravascularly.

Reverse transcriptase (RT)-PCR analysis of cytokine gene expression

Total RNAs were extracted from splenic lymphocytes of diabetic-prone BB rats treated with poly-I:C (0.05 and 5 μg/gm body weight) and saline by the acid guanidine thiocyanate–phenol–chloroform method [14]. Three micrograms of splenic total RNAs were converted to cDNAs using Superscript II (Life Technologies, Gaithersburg, MD, USA) and oligo (dT)₁₂–₁₈ (Life Technologies) in 20 μl of the reaction mixture at 42°C for 1 h. After the synthesis of cDNA, PCR was run using specific primers for IFN-γ, IL-2, IL-4, IL-10 and TNF as previously described [15]. Hypoxanthine phosphoribosyltransferase (HPRT) was used as an internal standard. The PCR condition was optimized for each set of primers. PCR was performed using a different number of cycles to ensure that amplification occurred in a linear range. The PCR mixture (50 μl) contained 0.2 mM concentration of each deoxynucleotide triphosphate, 1 μM concentration of each primer, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-Cl (pH 9.0) and 2.5 units of Taq polymerase (Pharmacia, Uppsala, Sweden). After amplification, the products were subjected to electrophoresis on 2% agarose gel and detected by ethidium bromide staining.

Statistical analysis

The product limit method of Kaplan and Meier was used to estimate survival from diabetes. Gehan’s Wilcoxon test compared the product limit functions. Group means were analysed by the Student’s t-test. Incidences of diabetes was compared by chi-square analysis with Yates’ correction.

Results

Effect of poly I:C administration on the development of diabetes

Survival from diabetes development over the entire 130 day observation period was lower in poly-0.05 treated BB rats when compared to saline treated control animals by survival curve analysis (P<0.01) (Figure 1). Treatment with poly-0.1 also decreased the development of diabetes after 76 days of age (P<0.04). By 130 days, the cumulative incidence of diabetes was lower in poly-0.05 treated than saline treated BB rats (P<0.04). Further, the mean (±SD) age (days) of diabetes onset was higher in poly-0.05 treated (87 days±13) when compared to saline treated (75.7 days±7.4) (P<0.02) and poly-0.1 treated rats (68.9 days±6.5) (P<0.02). Four non-diabetic poly-0.1 treated BB rats were followed for four additional months beyond the 130 day time point and were all found to remain non-diabetic.
Animal weights

The mean weights of animals from each treatment group at all time points prior to the development of diabetes were similar in poly I:C and saline treated rats (data not shown).

Pancreatic histopathology

Very little inflammatory response was present within the exocrine tissue in both poly I:C and saline treated rats. Inflammatory cells within the islets were predominantly mononuclear in all animals although some polymorphonuclear cells were found, particularly in islets from poly-0.1 treated rats. In general, pancreata from poly I:C administered BB rats were found to have less inflammation than pancreata from control animals (Figure 2). The degree of insulitis in saline and poly I:C treated BB rats is depicted in Table 1. The mean islet-inflammatory scores from randomly chosen 130 day old non-diabetic animals, treated with either poly-0.1 (n=3) or poly-0.05 (n=4) were lower than in six randomly chosen saline treated rats (P<0.005 and P<0.0001).

Flow cytometric analysis of PBMC phenotypes and mononuclear splenocyte class I expression

The phenotypes of PBMC harvested from five 130-day old non-diabetic poly-0.05 treated and control animals were determined. The proportions of PBMC with the NK cell phenotype (OX19−, OX8+), cytotoxic/suppressor T-cell phenotype (OX19+OX8+), and helper/inducer T-cell phenotype (OX19+, OX8−) in the poly-0.05 treated and non-diabetic control animals were similar (22.3% vs. 26.5%, 0.4% vs. 0.3%, and 4.7% vs. 2.6%). In addition, very few RT6+ cells were found (<0.5%) in both poly I:C treated and control rats. The degree of class I MHC expression on mononuclear splenocytes from BB rats following poly-0.05 (n=4), poly-5.0 (n=4), and saline (n=4) administration was compared. The mean (±SEM) fluorescence of cells from poly-0.05 treated animals was greater than that found in cells from saline administered control animals (91.8±8.5 vs 54.9±3.2, P<0.02), but not different to the mean fluorescence of cells from poly-5.0 treated animals (103.5±20).

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Figure 1. Incidence of diabetes mellitus in BB rats administered saline (n=22), poly I:C 0.05 µg/gm body weight (n=11), and poly I:C 0.1 µg/gm body weight (n=16) i.p., three times per week commencing at 35-40 days of age for 5 weeks. (–poly–) saline, (–poly-0.05, (–poly-0.1).

Figure 2. Representative hematoxylin and eosin stained pancreata (×360) from BB rats administered poly I:C (0.05 µg/gm body weight) (A) and saline (B). A small quantity of islet mononuclear cell infiltration was observed in poly I:C treated mice (A), while marked insulitis and islet destruction was present in control animals (B).

Table 1. Inflammatory scores (±SD) of islets from diabetes-prone BB rats treated with saline (n=6), poly-0.05 (n=4), and poly-0.1 (n=3)

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>P value vs. saline</th>
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</thead>
<tbody>
<tr>
<td>Saline</td>
<td>2.26</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Poly-0.05</td>
<td>0.32</td>
<td>0.16</td>
<td>0.001</td>
</tr>
<tr>
<td>Poly-0.1</td>
<td>0.61</td>
<td>0.47</td>
<td>0.005</td>
</tr>
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</table>

The degree of insulitis was scored as follows: 0 if no inflammation, 1+ if 1–10% of the islet is infiltrated, 2+ if 10–25%, 3+ if 25–75%, and 4+ for >75% islet involvement or islet fibrosis.
White blood cell count and differential

Peripheral blood was collected from BB rats in each treatment group (saline n=6, poly-0.05 n=7, poly-0.1 n=8), 3 weeks after commencing therapy to measure the mean total white blood cell count and differential (Table 2). These measurements were similar in the three treatment groups, with the exception of the granulocyte count which was significantly lower in the poly-0.1 treated rats when compared to saline treated control rats (P<0.02).

Induction of non-specific suppressor cell activity

To determine whether poly-I:C administration induces non-specific suppressor cell activity in BB rats, we compared the ability of splenocytes from five 130-day-old non-diabetic poly-0.05 treated rats with four saline age-matched non-diabetic control rats, to suppress responder cell proliferation to Con A (Figure 3). With no diabetes-prone BB rat spleen cells present, Con A (2 μg/ml) stimulated an increase of more than five-fold in ³H-thymidine incorporation over control responder cells (media without Con A). Notably, at a suppressor:responder splenocyte ratio of 0.5:1, only splenocytes from poly-0.05 treated animals were able to inhibit Con-A-induced ³H-thymidine incorporation into responder cells. At a higher suppressor:responder cell ratio (1:1), cells from poly I/C and control animals suppressed the proliferative response of responder splenocytes to Con A (P<0.05 and P<0.001).

Suppressor-cell function of mononuclear splenocytes from poly-0.05 (n=4), poly-5.0 (n=4) and saline (n=5) treated rats was also assessed by measuring the suppression of the T-cell response to alloantigen (Figure 4). At suppressor:responder cell ratios of 0.5:1 and 1:1, responder cell proliferation to allogeneic cells was 48% (P<0.04) and 84% (P<0.01) lower when exposed to mononuclear spleen-cells from poly-0.05 treated rats than when exposed to spleen cells from saline-treated rats. However, mononuclear spleen-cells from poly-5.0 treated animals had similar effects on responder-cell proliferation as spleen cells from saline-treated animals.

Table 2. Peripheral blood leukocyte counts in BB rats treated with saline, poly-0.01 or poly-0.05

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Saline (n=6)</th>
<th>Poly-0.1 (n=8)</th>
<th>Poly-0.05 (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total leukocytes</td>
<td>6883±1701</td>
<td>5399±912</td>
<td>7803±901</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1817±249</td>
<td>1836±290</td>
<td>2786±447</td>
</tr>
<tr>
<td>Atypical lymphocytes</td>
<td>184±61</td>
<td>169±45</td>
<td>245±111</td>
</tr>
<tr>
<td>Monocytes</td>
<td>1012±332</td>
<td>843±101</td>
<td>758±148</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>3838±371</td>
<td>2551±243*</td>
<td>4011±382</td>
</tr>
</tbody>
</table>

Diabetes-prone BB rats were administered saline, poly I/C 0.1 μg/gm body weight, or poly I/C 0.05 μg/gm body weight i.p. three times per week commencing at 40 days of age. Peripheral blood leukocyte counts (cells/μL whole blood) ±SE are depicted. *P<0.02 vs saline.

Spleen cells from rats administered poly-0.05 inhibit the development of diabetes

To determine whether the diabetes-sparing activity of poly I/C-0.05 is mediated by the induction of immunoregulatory cells with suppressor-like activity,
the development of diabetes was compared in BB rats injected with spleen cells from poly I:C and saline treated animals, as shown in Figure 5. The development of diabetes was found to have decreased in recipient animals receiving cells from poly-I:C-treated rats (P<0.004).

In vitro effect of poly I:C on Con-A-stimulated splenocytes

To further explore the effect of poly I:C on T-cell responses, the in vitro effect of poly I:C on Con-A-induced proliferative responses of splenocytes from diabetes-prone BB rats was investigated (Figure 6). Pre-incubation of diabetes-prone BB rat spleen-cells with 0.05 and 0.5 μg/ml concentrations of poly I:C led to significant decreases in the proliferative response of cells to Con A (35% and 66%).

Cytokine gene expression

To assess which cytokines may be important to the diabetes-sparing effect of low dose poly I:C administration, we determined the cytokine mRNA content in spleen cells of poly-0.05, poly-5.0, and saline-treated BB rats (Figure 7). TNF-α mRNA and IL-10 mRNA content was found to be lower in spleen cells in low dose poly-I:C-injected rats than in controls. High dose poly-I:C-administration increased the levels of TNF-α mRNA and did not alter the expression of IL-10. IL-2 mRNA, IL-4 mRNA and IFN-γ mRNA levels were unaltered in poly-0.05 and poly-5.0 administered rats.

Discussion

It has been reported previously that treatment of diabetes-prone BB rats with high-dose poly I:C (5 μg/gm body weight) accelerates the development of diabetes [8, 9]. We now demonstrate that the administration of 50 (poly-0.1) and 100 (poly-0.05)-fold lower poly-I:C-dosages increases the rate of survival from diabetes in BB rats; poly-0.05 also increased the mean age of diabetes onset. The diabetes-sparing
effect of poly I:C was very long lasting. All four non-diabetic poly-0.1 treated animals that were followed for an additional 4 months of observation remained non-diabetic.

Poly I:C administration did not appear to be overtly toxic to the rats. All poly-I:C treated animals gained weight at the same rate as control animals and exhibited no abnormal behaviour patterns.

The islet inflammatory scores of non-diabetic, 130-day old poly-0.05 treated rats were lower than those found in the saline treated (mostly diabetic) control animals. This suggests that the diabetes-sparing effect of poly-0.05 is mediated by inhibition of the insulitis which causes diabetes. This inhibition may affect either the recruitment or the initial activation of immune cells.

Although peritoneal inflammation could potentially alter the development of diabetes, no evidence of significant peritonitis was found upon surgical exploration of the abdominal cavity, or following, microscopic analysis of the pancreata and peritoneal washings obtained from four poly-0.05, and four saline treated rats immediately after treatment (data not shown).

Autoimmune disorders are thought to result from an imbalance between regulatory-cell and effector cell activities. Effector cells such as T cells [16] and NK cells [17] are reported to play an important role in the development of diabetes in the BB rat. Several strategies have been attempted to suppress or eliminate effector cells, and in turn prevent diabetes. These treatments have been limited due to the state of generalized immunosuppression that they induce. These therapies include the utilization of immunosuppressive drugs such as glucocorticoids, cyclosporin, anti-lymphocyte serum, FK 506, and T-cell antibodies [18–21], as well as inhibitors of macrophage function such as silica [21]. In the present report, however, the lack of suppression of the total white blood cell count, any specific peripheral blood leukocyte as examined on peripheral smear, or of any specific mononuclear leukocyte phenotype as measured by flow cytometry following poly I:C administration does not support, although does not rule out, a generalized immunosuppressant basis of poly I:C action.

Poly I:C administration increased the peripheral blood granulocyte count, a property of poly I:C previously recognized in humans. However, because this effect was present only with the higher poly I:C dose (poly-0.1) which was less effective in preventing diabetes than the lower dosage, we suspect the increase in granulocyte count may not play an important role in inhibiting the diabetic process.

Augmentation of immunoregulatory cells by immunostimulation has been hypothesized to provide a future basis upon which IDDM could be prevented [23]. Since poly I:C is an immunostimulant known to increase NK cell activity, and levels of various cytokines [24–27], its diabetes-sparing activity was hypothesized to be mediated by the induction of regulatory cells. The data described here support this hypothesis; splenocytes from poly-0.05 treated BB rats inhibit responder cell proliferation to Con A and alloantigen to a greater degree than splenocytes from saline treated animals.

The strongest evidence that the induction of immunoregulatory cells mediates the diabetes sparing effect of poly-0.05 comes from the demonstration that poly-0.05 administration induces mononuclear leukocytes that suppress the development of diabetes when injected into BB rats. Poly-0.05 administration may correct the depressed regulatory cell activity thought to play a role in the pathogenesis of diabetes. The increased expression of class-I MHC molecules on mononuclear leukocytes by poly-0.05 could play a role in the induction of immunoregulatory cells and diabetes-sparing activity of poly-0.05, since increased class I MHC expression has been shown to augment the class-I MHC restricted induction of suppressor T-cells [28]. The specific regulatory-cell which may be augmented is yet to be identified but our results suggest that it is not the R+ T-cell, a cell reported to exhibit suppressor activity [29, 30]. Since IFN-α is induced by poly I:C, increases class-I MHC expression, and has been shown to induce suppressor cell activity [31], IFN-α may play a role in the augmentation of regulatory cell activity.

In the NOD mouse model of IDDM, poly I:C administration potently prevents the development of diabetes [7]. This diabetes-sparing activity of poly I:C is also associated with the recruitment of cells which suppress alloantigen induced T-cell responses [7]. Thus, the mechanism of poly-I:C action to prevent diabetes may be similar in the BB rat and NOD mouse.

The administration of another immunostimulant, complete Freund’s adjuvant (CFA), has recently been reported to inhibit the development of insulitis and diabetes in BB rats [32]. Analogous to the data presented here, CFA adjuvant induces non-specific suppressor-cell activity, as determined by the inhibition of lymphoproliferative responses of diabetes-resistant BB splenocytes to lipopolysaccharide and allogeneic spleen cells [33]. However, in contrast to this report, the abrogation of diabetes with CFA requires the initiation of treatment at an earlier age than that required with poly I:C. This suggests that the mechanisms of diabetes abrogation of poly-0.05 and CFA administration are different.

We have reported previously that poly I:C administration accelerates diabetes in BB rats [8, 9], while the present report describes the inhibition of the diabetic process by poly I:C. These disparate data appear to be due to the different dosages of poly I:C. Other than the use of a 100-fold lower poly I:C dosage, all other treatment parameters, including drug lot, age at initiation of drug treatment, and the frequency and route of poly I:C administration were the same in this report as in our previous study [8]. Several possibilities may underlie the surprising dose-dependent effects that we have observed. Since poly I:C is known to induce IFN-α, interferon-γ, interleukin (IL) 1, and granulocyte-macrophage colony stimulating factor [23–25, 34], it is possible that the different poly I:C dosages induce distinct cytokine profiles. These distinct cytokine profiles may in turn differentially affect
the immune mediated β-cell destructive process. Alternatively, the different dosages of poly I:C may result in the induction of differing amounts of a single cytokine, which in turn may have opposite effects on the development of diabetes; this latter phenomenon has been previously reported. The administration of higher dosages of IL-1 accelerates, while lower dosages inhibit the development of diabetes in the diabetes-prone BB rat [35].

The data described here suggest that the opposite effects induced by the different dosages of poly I:C on the diabetic process may, at least in part, be due to the differential induction of suppressor-cell activity. The suppressor activity of total mononuclear splenocytes, as assessed by in vitro inhibition of Con-A stimulated responder cells, was induced by low-dose poly I:C (poly-0.05) injections but not by high-dose poly I:C (poly-5.0) administration. The number of RT6+ mononuclear cells, a cell population with putative suppressor activity, were found in negligible quantities in the poly-0.05 and administered animals (data not shown) and thus do not appear to play a role. Also, the induction of MHC class-I expression cannot explain the diverse biological response of the different poly I:C doses, since high- and low-dose poly I:C induced similar class-I MHC expression in mononuclear leukocytes.

The role of tumour necrosis factor (TNF) in the pathogenesis of diabetes is controversial. TNF is thought to be important in the diabetic process for several reasons. TNF is cytotoxic to islets [36], endogenous TNF-α production is greater in NOD mice [37] and BB rats [38], TNF antibodies prevent diabetes in NOD mice [37], and TNF accelerates the diabetes onset in NOD mice [39]. There is, however, evidence to suggest that TNF has the opposite effect [40, 41]. Our data supports a pathogenic role for TNF, since low-dose poly I:C depresses and high dose poly I:C increases spleen cell TNF mRNA content. These differences in TNF expression with high and low doses of poly I:C may explain the opposing effects that high dose and low dose poly I:C treatments have on the development of diabetes.

The autoimmune diabetic process in the NOD mouse and humans is thought to be mediated, in part, by T-helper Th-1 cells, producers of IFN-γ and IL-2, while Th-2 cells, producers of IL-4 and IL-10, are thought to be protective [42]. We have found no evidence that low-dose poly I:C administration acts by reversing this increased Th 1/Th 2 activity. Our finding that low, but not high dose poly I:C treatment decrease IL-10 mRNA in spleen cells is consistent with the hypothesis that IL-10 plays a positive role in the diabetic process and low dose poly I:C inhibits this action. This mediating role of IL-10 in IDDM has been previously reported with the demonstration that the expression of IL-10 in the islets of transgenic NOD mice accelerates the onset of diabetes [43, 44]. This paradoxical effect of IL-10 on the diabetic process has been recently reviewed [45].

In conclusion, we demonstrate that low-dose poly I:C administration prevents the development of insulitis, and in turn diabetes in the diabetes-prone BB rat. This inhibition appears to be mediated by the induction of regulatory cells capable of suppressing T-cell responses to Con A and alloantigens, and inhibiting the development of diabetes. Immunostimulation with poly I:C may be useful in elucidating the pathogenesis of IDDM and providing a means to prevent it.

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Low dose poly I:C prevents diabetes


