Poly I:C Induces Development of Diabetes Mellitus in BB Rat

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Polyinosinic polycytidilic acid (poly I:C), an inducer of α-interferon, accelerates the development of diabetes in diabetes-prone (DP) BioBreeding (BB) rats. This study investigates the effect of administering poly I:C to a diabetes-resistant (DR) strain of BB rats. We compared the incidence of diabetes, the degree of insulitis, the number of NK cells, helper-inducer cells, cytotoxic-suppressor cells, Ia$^+$ T cells, RT6.1$^+$ T cells, and NK cell bioactivity in DR rats treated with saline and with a 5 μg/g body wt (poly-5) dose and a 10 μg/g body wt (poly-10) dose of poly I:C. The incidence of diabetes was also compared with that of DP rats receiving poly-5. We found that both doses of poly I:C significantly induce the development of diabetes in the DR BB rat. However, treatment of DR rats with the higher dose induces a greater rate of development of diabetes and earlier onset of diabetes than the lower poly-5 dose. The rate of diabetes development and the mean age of onset were similar in poly-10–treated DR and poly-5–treated DP rats. A significant degree of insulitis occurred in all the poly I:C–treated DR rats, even those not developing diabetes. Peripheral blood NK cell number was greater in poly I:C than in saline-treated rats, after 2 wk of treatment and when killed. The percentage of OX19$^+$ peripheral blood mononuclear cells expressing RT6.1 allotype or Ia antigen were similar in poly I:C– and saline-treated rats. We conclude that poly-10 administration alone can induce diabetes in 100% of DR rats, and although treatment with poly-5 is less effective, it causes significant insulitis in all treated rats. The similar mean time of onset and rate of development of diabetes in poly-10–treated DR rats and poly-5–treated DP rats is consistent with a similar mechanism of poly I:C action in the DR and DP BB rats. Although the specific mechanism is not defined, NK cell numbers are elevated with poly I:C treatment. Alterations in RT6.1$^+$ and Ia$^+$ T cells do not appear to play a role. *Diabetes* 41:515–20, 1992

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The BB/Wor rat has been used as an animal model to study the pathogenesis of diabetes mellitus. As in the human, this rat has insulin deficiency and exhibits many signs of autoimmunity (1–3). These rats are lymphopenic and deficient of T cells expressing RT6.1, cells thought to play a regulatory role in suppressing the development of diabetes (4–6). Elevated Ia antigen-bearing T cells found in diabetes-prone (DP) BB rats and human insulin-dependent (type I) diabetic patients are thought to play a role in the pathogenesis of diabetes (7,8). Diabetes-resistant (DR) BB rats, a subline of the DP BB rat rarely develops diabetes (9), are neither lymphopenic nor deficient of T cells expressing RT6.1, cells thought to be important for their resistance to diabetes (5,10).

We recently demonstrated that polyinosinic polycytidilic acid (poly I:C), an inducer of interferon (11,12) and other cytokines (13,14), accelerates the onset of diabetes in DP BB rats (15) and supports a role of poly I:C–induced cytokines and possibly α-interferon (α-IFN) in the pathogenesis of diabetes in DP BB rats.

Recently, Thomas et al. (16) reported that the administration of poly I:C induces diabetes in the DR BB rat. The depletion of RT6$^+$ T cells or exposure of DR BB rats to viral pathogens were required for full diabetogenic effect of poly I:C, implicating the importance of RT6$^+$ T cells and environmental factors in the pathogenesis of diabetes. Conceivably, the administration of a higher dose of poly I:C alone may not require the depletion of RT6$^+$ T cells or exposure of viral pathogens to induce a high frequency of diabetes and in turn may lead to a

further understanding of its pathogenesis. The importance of NK and Ia$^+$ T cells as immunomodulators of poly I:C–induced diabetes in DR BB rats has also not been studied.

This report further characterizes the model of poly I:C induction of diabetes in DR BB rats with similar and higher dosages of poly I:C than previously examined. The effect of poly I:C treatment on NK cell number and activity, T cells expressing RT6.1 and Ia antigens, and pancreatic histopathology of DR BB rats were also investigated.

RESEARCH DESIGN AND METHODS

Animals. DR and DP BB/Wor rats were obtained from the viral antibody–free (VAF) colony at the Univ. of Massachusetts. A special viral-free environment was maintained by autoclaving bedding and food, providing acidified water, and maintaining cages in a laminar flow hood with filtered bonnets. Viral serology screening for antibodies to Sendai virus, sialodacryoadenitis virus, Kilham's rat virus, and Toolan's H-1 virus were negative and in random samples in six DR BB rats and eight sentinal animals.

Experimental design. Rats were randomly assigned to different treatment groups. At an average of 40 days old, DR BB rats were injected three times/week with either saline (i.p.; n = 12), 5 μg/g body wt i.p. poly I:C (poly-5; n = 11), or 10 μg/g body wt i.p. poly I:C (poly-10; n = 7). Ten DP BB rats were similarly injected with poly-5. Rats were diabetic when blood glucose levels, determined three times/week, were > 250 mg on 2 consecutive days. Animals were sacrificed when diagnosed with diabetes or at 120 days in nondiabetic rats. Cell surface phenotypes of peripheral blood mononuclear cells (PBMNCs) were determined in a subset of rats after 2 wk of treatment and when killed. NK cell activity of splenic leukocytes and RT6 and Ia expression on peripheral T cells were determined at sacrifice.

Paraffin sections of pancreas were hematoxylin and eosin stained and examined under light microscopy. The degree of islet inflammation was scored as follows: 0, no inflammation; 1+, 1–10% of the islet involved; 2+, 10–25%; 3+, for 25–75%; and 4+, >75% or fibrosis of the islet. The mean of at least six islets/rat were examined.

PBMNCs were separated from whole blood with Immunolyse (Coulter, Hialeah, FL) for most analysis and Ficoll-hypaque (1.077) centrifugation for analysis of RT6.1 expression. Phenotypes of 5000 mononuclear cells were analyzed by flow cytometry (fluorescence-activated cell sorter [FACS], FACStar Plus, Becton Dickinson, Rutherford, NJ) with the following monoclonal antibodies: OX19 (Pan T) FITC-conjugated (Serotec, Oxford, UK), OX8 (cytotoxic-suppressor cell, NK) phycoerythrin (PE) conjugated (Serotec), OX6 (monomorphic determinant on Ia and B cells) PE-conjugated (Serotec), and monoclonal antibody (MoAb) 3.2.3 (NK cell) conjugated to FITC (a gift from Hiserodt (Univ. of Pittsburgh, Pittsburgh, PA); 17). 17). Two-color fluorescence analysis defined OX19$^+$ OX8$^+$ cells as cytotoxic-suppressor cells, OX19$^+$ OX8$^-$ as helper-inducer cells, and OX19$^-$OX8$^+$ as NK cells. NK cells were also defined with MoAb 3.2.3. OX19$^+$ OX6$^+$ cells were defined as Ia$^+$ T cells. RT6.1 expression on T cells was assessed with a rat MoAb (DS4.23; a gift from D. Lubaroff (Univ. of Iowa, Iowa City, IA); 18). A PE-labeled F(ab')$_2$ goat anti-rat IgG (Serotec) was used as secondary antibody.

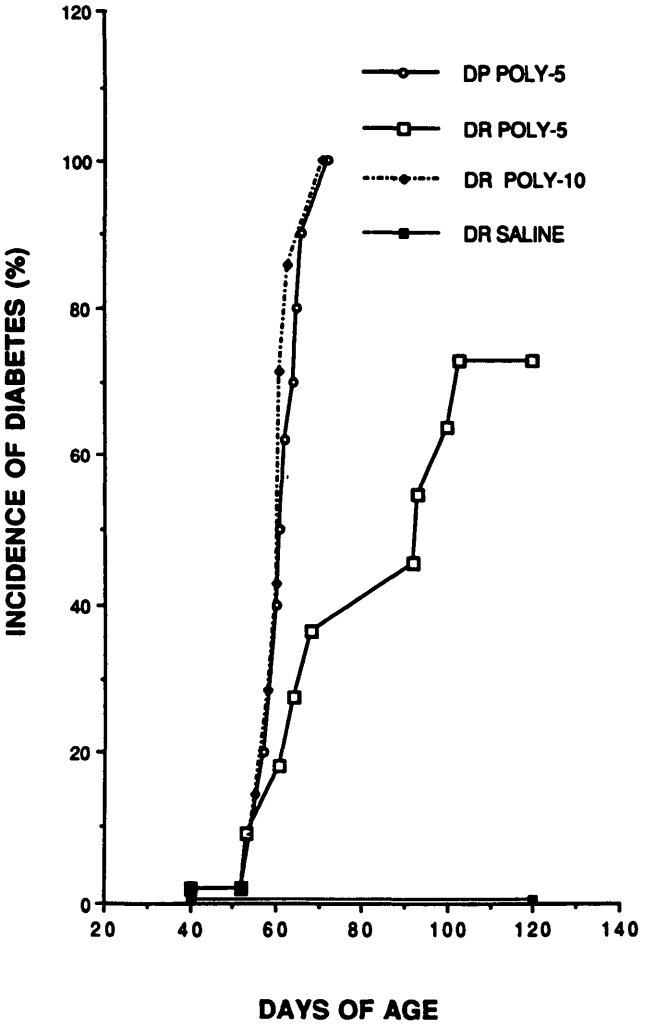

FIG. 1. Incidence of diabetes after administration of saline (n = 12), 5 μg/g polyinosinic polycytidilic acid (poly-5; n = 11) and poly-10 (n = 7) to diabetes-resistant (DR) BB rats and poly-5 (n = 10) to diabetes-prone (DP) rats.

NK cell bioactivity of splenic mononuclear leukocytes was assessed by a standard ^{51}Cr microcytotoxicity release assay with the YAC-1 cells as targets (19).

The incidence of diabetes of test groups were compared by Fisher's exact test. Group means were compared by analysis of variance. The product-limit method of Kaplan and Meier was used to estimate survival (from diabetes) function. Gehan's Wilcoxon test compared the product-limit functions.

RESULTS

No saline-treated DR BB rats developed diabetes. However, 73 and 100% of the DR rats developed diabetes in the poly-5 and poly-10 treatment groups, respectively (Fig. 1). By 34 days of treatment, all poly-10 DR and

TABLE 1
Comparisons of rates of diabetes development with survival-curve analysis

BB rat	Treatment	*Z* scores	*P* value
DR	saline vs. DR poly-5	3.474	0.001
DR	saline vs. DR poly-10	4.104	0.001
DR	poly-5 vs. DR poly-10	2.410	0.016
DP	poly-5 vs. DR poly-5	2.470	0.013
DP	poly-5 vs. DR poly-10	0.589	0.556

DR, diabetes resistant; poly-5, 5 μg/g body wt polyinosinic polycytidilic acid (poly I:C); poly-10, 10 μg/g body wt poly I:P; DP, diabetes prone.

poly-5 DP treated rats exhibited diabetes. The incidences of diabetes in the poly-5 DR and poly-10 DP treated rats became different ($P < 0.01$) by 71 days of age. When comparing survival analysis curves, the development of diabetes was greater in DR rats treated with either dose of poly I:C than saline and in DR rats treated with poly-10 than with poly-5 (Fig. 1, Table 1). The development of diabetes was greater in DP than in DR rats treated with poly-5 but almost identical to that of poly-10 treated DR BB rats (Fig. 1, Table 1). The mean age of onset of diabetes was younger in the DR BB rats

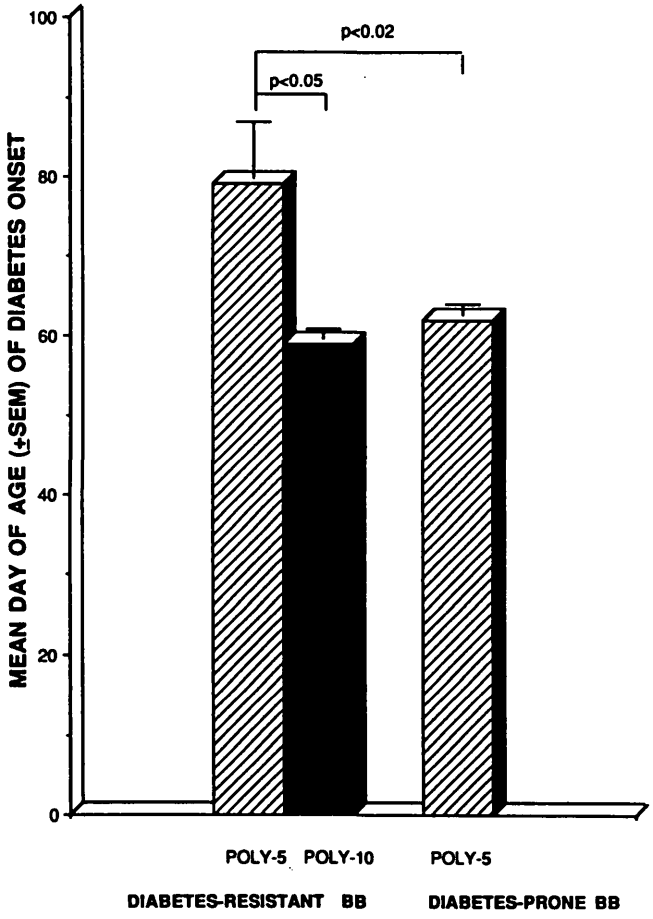

FIG. 2. Mean age of diabetes onset in diabetes-resistant BB rats treated with 5 μg/g polyinosinic polycytidilic acid (poly-5) and poly-10 and in diabetes-prone rats treated with poly-5. No saline-treated rats developed diabetes.

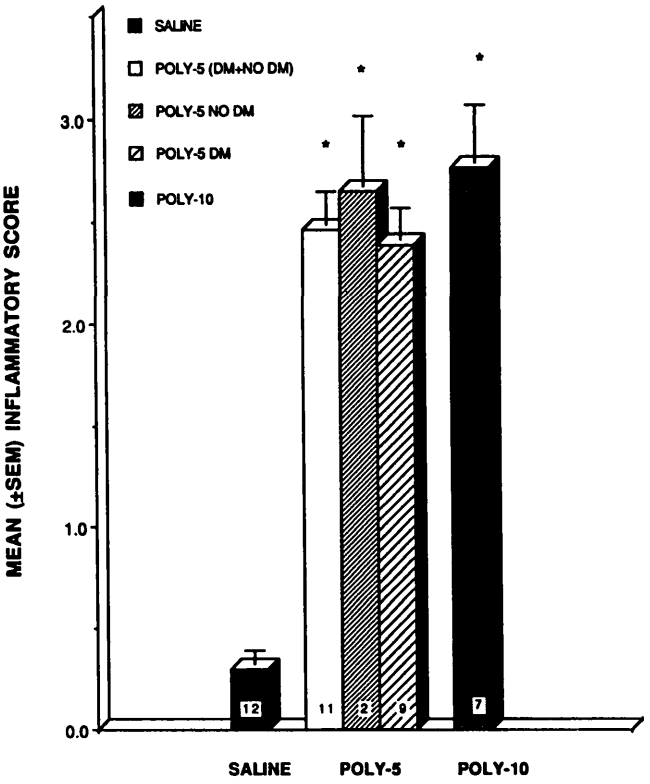

FIG. 3. Degree of mononuclear cell islet infiltration as assessed by the inflammatory score (see METHODS) of diabetes-resistant BB rats treated with saline, 5 μg/g polyinosinic polycytidilic acid (poly-5), and poly-10. Mean ± SE inflammatory score of poly-5–treated rats developing diabetes (poly-5 DM) and not developing diabetes (poly-5 NO DM) is depicted. *$P < 0.001$.

treated with the higher dose of poly I:C (Fig. 2). The mean age of diabetes onset of DP rats treated with poly-5 was similar to that of DR BB rats treated with poly-10 and less than the mean age of diabetes onset of DR rats administered poly-5 ($P < 0.02$). The rate of diabetes development (percentage of total rats developing diabetes per day) was similar in poly-10–treated DR rats and poly-5–treated DP rats (5.64 vs 5.60% of total rats/day) and was more than twice as great than that of poly-5–treated DR BB rats.

Mean body weights of all groups were not significantly different before treatment. However, the mean body weight of the poly-10 group was less than saline-treated controls after 10 and 17 days of treatment ($P < 0.05$).

Histopathology. In general, islets of saline-treated DR BB rats exhibited very little or no inflammation. A significant inflammatory reaction, predominantly of mononuclear cells, was noted in 100% of the poly I:C treated rats. Some rats exhibited an inflammatory cell involvement within the perivascular and interlobular spaces. A mild inflammatory response without necrosis was occasionally present within the exocrine tissue.

The mean islet inflammatory score of the poly-5 and poly-10 DR rats were significantly greater than saline-treated controls (Fig. 3). The islet inflammatory score of the poly-5 DR rats not developing diabetes was greater than saline-treated controls and no different from poly I:C–treated DR rats developing diabetes.

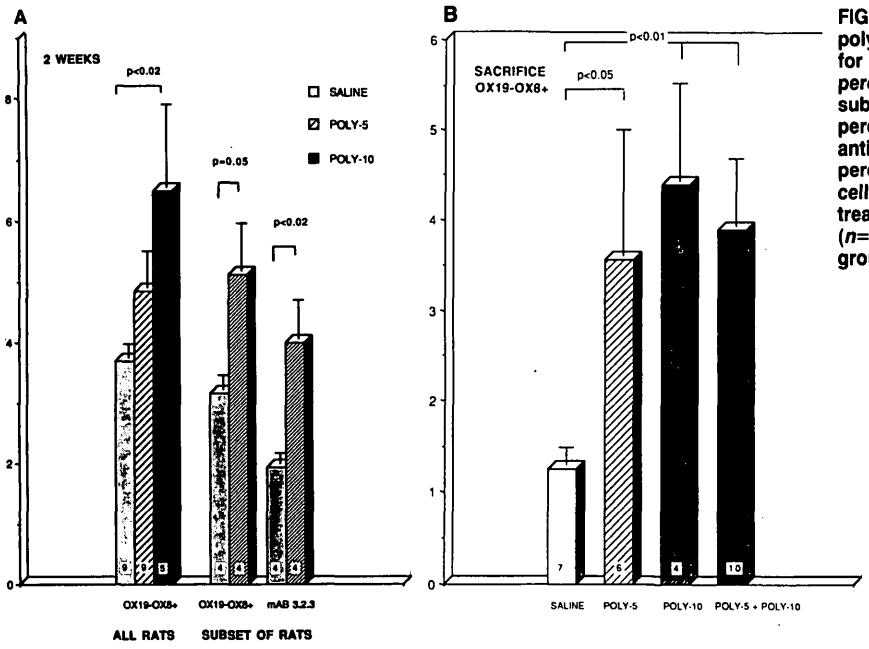

FIG. 4. *A*: **effect of administering 5 μg/g polyinosinic polycytidilic acid (poly-5) and poly-10 for 2 wk to diabetes-resistant (DR) BB rats on the percentage of peripheral $OX19^-OX8^+$ cells. In a subset of saline- and poly-5–treated rats, the percentage of $OX19^-OX8^+$ and monoclonal antibody (mAb) 3.2.3$^+$ cells are depicted.** *B*: **percentage of $OX19^-OX8^+$ peripheral mononuclear cells present at time of killing of DR BB rats treated with saline (n=7), poly-5 (n=6), and poly-10 (n=4). Combined data from poly-5 and poly-10 groups are also depicted. NK, natural killer.**

Flow cytometry. The percentage of $OX19^-OX8^+$ T cells (NK cell) was higher in the poly 10 than saline-treated group (Fig. 4). In a subset of rats, poly I:C increased the NK cell number by >100% ($P < 0.02$) with the 3.2.3 MoAb method and 60% with the two-color method ($P = 0.05$). When the rats were killed, NK cell number remained elevated in poly I:C–treated DR rats. The number of $OX19^+OX8^-$ (helper-inducer) and $OX19^+$ $OX8^+$ (cytotoxic-suppressor) cells were similar in saline- and poly I:C–treated groups at 2 wk and at death (data not shown).

The mean ± SE percentage of T cells expressing RT6.1 were similar in saline-treated (65.4 ± 2.6%) and poly-5–treated (62.4 ± 3.4%) groups. The percentage of T cells expressing Ia^+ in seven saline and six poly-5–administered DR rats were similar (1.3 and 1.5%, respectively).

When killed, the NK cell activity of splenic mononuclear cells of BB DR rats administered poly I:C and saline were no different (data not shown).

DISCUSSION

We have previously established a model of accelerated diabetes in the DP BB rat after the administration of poly I:C. Based on these observations, Thomas et al. (16) described the induction of diabetes in DR BB rats. The results of this report confirm and extend the findings of Thomas with a similar and higher dosage of poly I:C than previously examined. The effect of poly I:C is dose dependent. The administration of the higher dose causes a greater rate of development and incidence of diabetes and induces diabetes at a younger mean age than the lower dose. The incidence of diabetes after 30 days of poly-5 treatment in this report is similar to that described by Thomas et al. (36 vs 22%); 16). In this report, in which the duration of poly-5 administration was extended from 30 to 80 days, there is a much greater final incidence of diabetes (73%). The mean age of diabetes onset and rate of development of diabetes in poly-10–treated DR BB rats and poly-5–treated DP BB rats are similar and consistent with a similar mechanism of action of poly I:C in both types of BB rats.

All DR rats treated with poly I:C developed a significant mononuclear infiltration of the islets similar to that found in DP rats treated with poly I:C and those spontaneously developing diabetes (15,20). Mild inflammatory changes occasionally occurred within the exocrine tissue and were more common in DR BB rats treated with the higher dose of poly I:C. These changes are also present in diabetic DP rats and DR BB rats developing diabetes spontaneously or after the administration of RT6.1 antibody (20,21). However, the interlobular infiltration previously described in poly I:C–treated DP BB rats (15) was much less prominent in the poly-10–treated DR rat pancreas and is not generally present in rats spontaneously developing diabetes.

The regulatory role of $RT6^+$ T cells in the autoimmune development of diabetes has been supported by the findings that a deficiency of $RT6^+$ T cells is present in DP BB rats and not DR BB rats (10), the depletion of $RT6^+$ T cells in conventionally housed DR BB rats induces diabetes (22), and that T cells expressing RT6 may play an important role in preventing diabetes in DP BB rats transfused with spleen cells of DR BB or Wistar-Furth rats (23,24).

In a previous study, depletion of $RT6.1^+$ T cells in the VAF BB rat permits poly-5 treatment to induce a higher incidence of diabetes (94 vs. 22%; 16). In this study, poly I:C administration at twice the previously studied dose over the same observation period can alone, without $RT6^+$ T cell depletion, render 100% of DR BB rats diabetic. We conclude that the higher dose of poly I:C accounts for these results because we obtained a similar incidence of diabetes as Thomas et al. (16) with the same lower dosage with a similar observation time.

Our finding that poly I:C does not alter the number of

RT6.1$^+$ peripheral T cells extends the results of Thomas et al. (16) who examined lymph node cells and suggested that poly I:C–induced diabetes is not mediated by changes in RT6$^+$ T cells (16). Likewise, because poly I:C administration does not alter levels of cytotoxic-suppressor, helper-inducer, or Ia$^+$ cells, these cells do not appear to play a pathogenetic role in poly I:C–induced diabetes in the DR rat.

Although the precise immune mechanism of poly I:C is unknown, we speculate that poly I:C administration dose dependently induces cytokine or cytokines, perhaps those induced by viral infection (e.g., α-IFN), that augment effector cell function and override regulatory cell inhibition. The higher incidence of diabetes in viral seropositive BB rats than in seronegative DR BB rats treated with poly-5 (16) further supports the importance of viral-induced cytokines. The connection between viral-induced cytokines and diabetes in the BB rat has also previously been suggested by the finding that viral seropositive and not seronegative DR rats develop diabetes after RT6 depletion. The induction of similar cytokines by poly I:C may also explain why RT6 depletion may also induce diabetes in poly I:C–treated DR rats (16).

NK cells are reported to play an important role in the pathogenesis of spontaneous diabetes in DP BB rats (25,26). Increased peripheral NK cell number in poly I:C–treated DR BB rats at 2 wk and when killed is consistent with, but does not prove, a role of NK cells in the mechanism of poly I:C–induced diabetes. However, peripheral NK cell number may not reflect NK activity in the islets. Also, the small absolute difference between the groups is difficult to assess considering the inherent errors of two-color flow cytometric analysis. However, the difference of means appears to be real because it was statistically different and present when the two-color and single-color flow cytometric methods were used. Although unaltered when killed, NK bioactivity of splenic cells at this time may not as well reflect the pathogenetic process that occurs at an earlier time.

The specific mechanism of poly I:C–mediated augmentation of NK cells and induction of diabetes is unknown. However, poly I:C has been previously demonstrated to increase NK cell number in rodents (27,28). The mechanism of this activity may be attributed to the induction of α-IFN that occurs in poly I:C–treated rats (11), including DP BB rats (15), and which in turn augments NK cell number (28) and activity (29). Various reports describing poly I:C and α-IFN induction of other cytokines, e.g., interleukin-1, -2, and -6, γ-interferon, colony-stimulating factors (12,14,30,31), and macrophage activity (32), suggest other possible mechanisms for the increase in NK cell number and induction of diabetes in poly I:C–treated DR BB rats. These specific mechanisms of action of poly I:C are subjects for future investigations.

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REFERENCES

1. Nakhooda AF, Like AA, Chappel CL, Murray FT, Marliss EB: The spontaneously diabetic Wistar rat: metabolic and morphologic studies. *Diabetes* 26:100–12, 1976
2. Mordes JP, Desemone J, Rossini A: The BB rat. *Diabetes Metab Rev* 3:725–50, 1987
3. Like AA, Anthony M, Guberski DL, Rossini AA: Spontaneous diabetes mellitus in the BB/W rat: effects of glucocorticoids, cyclosporin-A and antiserum to rat lymphocytes. *Diabetes* 32:326–30, 1983
4. Jackson R, Rossi N, Crump T, Haynes B, Eisenbarth GS: The BB diabetic rat: profound T cell lymphopenia. *Diabetes* 30:887–89, 1982
5. Greiner DL, Handler ES, Nakano K, Mordes JP, Rossini AA: Absence of the RT-6 T cell subset in diabetes prone BB/W rats. *J Immunol* 136:148–51, 1986
6. Lang F, Kastern W: The gene for the T lymphocyte alloantigen RT6, is not linked to either diabetes or lymphopenia and is not defective in the BB rat. *Eur J Immunol* 19:1785–89, 1989
7. Francfort JW, Barker CF, Kimura H, Silvers WK, Frohman M, Naji A: Increased incidence of Ia antigen-bearing T lymphocytes in the spontaneously diabetic BB rat. *J Immunol* 134:1577–81, 1985
8. Jackson RA, Morris MA, Haynes BF, Eisenbarth GS: Increased circulating Ia-antigen bearing T cells in type I diabetes mellitus. *N Engl J Med* 306:785–88, 1982
9. Butler L, Guberski DL, Like AA: Genetics of diabetes production in the Worcester colony of the BB rat. In *Frontiers in Diabetes Research: Lessons from Animal Diabetes*. Vol. 2. Shafrir E, Renold AE, Eds. London, UK, Libbey, 1988, p. 74–78
10. Like AA, Guberski DL, Butler L: Diabetic BioBreeding/Worcester (BB/Wor) rats need not be lymphopenic. *J Immunol* 136:3254–58, 1986
11. DeClercq E: Interferon induction by polynucleotides, modified polynucleotides and polycarboxylates. In *Methods in Enzymology*. Vol. 78, Petska S, Ed. New York, Academic, 1987, p. 227–36
12. duBuy HG, Johnson ML, Buckler CE, Baron S: Relationship between dose size and dose interval of polyinosinic polycytidilic acid and interferon hyporesponsiveness in mice. *Proc Soc Exp Biol Med* 135:340–44, 1970
13. Tamura-Nishimura M, Sasukawa S: The roles of protein kinase C and cyclic nucleotide dependent kinase in signal transduction in human interferon-gamma induction by poly I:poly C. *FEBS Lett* 261:343–46, 1990
14. Weissenbach J, Chernajovsky Y, Zeevi M, Shulman L, Soreq H, Nir V, Wallach D, Perricaudet M, Tiollais P, Revel M: Two interferon mRNAs in human fibroblasts: in vitro translation and escherichia coli cloning studies. *Proc Natl Acad Sci USA* 77:7152–56, 1980
15. Ewel C, Sobel DO, Zeligs B, Abbassi V, Bellanti J: The role of alpha interferon in the pathogenesis of diabetes mellitus (Abstract). *Diabetes* 38 (Suppl. 2):73A, 1989
16. Thomas VA, Woda BA, Handler ES, Greiner DL, Mordes JP, Rossini AA: Altered expression of diabetes in BB/Wor rats by exposure to viral pathogens. *Diabetes* 40:255–58, 1991
17. Chambers WH, Vujanovic NL, DeLeo AB, Olszowy MW, Herberman RB, Hiserodt J: Monoclonal antibody to a triggering structure expressed on rat natural killer cells and adherent lymphokine-activated killer cells. *J Exp Med* 169:1373–89, 1989
18. Ely JM, Greiner DL, Lubaroff DM, Fitch FW: Characterization of mono-clonal antibodies that define rat T cell alloantigens. *J Immunol* 130:2798–8203, 1983
19. Reynolds CW, Timonen T, Herberman RB: Natural killer (NK) cell activity in the rat. I. Isolation and characterization of the effector cell. *J Immunol* 127:282–87, 1981
20. Seemayer TA, Tannenbaum GS, Goldman HY, Colle E: Dynamic time course studies of the spontaneously diabetic BB Wistar rat. *Am J Pathol* 106:237–49, 1982
21. Like AA: Depletion of RT6.1$^+$ T lymphocytes alone is insufficient to induce diabetes in diabetes resistant BB/Wor rats. *Am J Pathol* 136:565–74, 1990
22. Greiner DL, Mordes JP, Handler ES, Angelilo M, Nakamura N, Rossini AA: Depletion of RT6.1$^+$ T lymphocytes induces diabetes in resistant BioBreeding Worcester (BB/W) rats. *J Exp Med* 166:461–75, 1987
23. Rossini AA, Mordes JP, Greiner DL, Nakano K, Appel MC, Handler ES: Spleen cell transfusion in the BB/W rat: prevention of diabetes,

MHC restriction and long-term persistence of transfused cells. *J Clin Invest* 77:1399–401, 1986
24. Burstein D, Mordes JP, Greiner DL, Stein D, Nakamura N, Handler ES, Rossini AA: Prevention of diabetes in BB/Wor rat by single transfusion of spleen cells: parameters that affect degree of protection. *Diabetes* 38:24–30, 1989
25. Woda BA, Like AA, Padden CC, McFadden ML: Deficiency of phenotypic cytotoxic-suppressor T lymphocytes in the BB/W rat. *J Immunol* 136:856–59, 1987
26. Like AA, Biron CA, Weringer EJ, Byman K, Scroczynski E, Guberski DL: Prevention of diabetes in BioBreeding/Worcester rats with monoclonal antibodies that recognize T lymphocytes or natural killer cells. *J Exp Med* 164:1145–59, 1986
27. Testi R, Gali MC, Piccoli M, Herberman RB, Frati L, Santoni A: Sequential metabolic events and morphological changes during in vivo large granular lymphocyte activation and proliferation. *Cell Immunol* 102:78–88, 1980
28. Santoni A, Piccoli M, Ortaldo JR, Mason L, Wiltrout RH, Herberman RB: Changes in number and density of larger granular lymphocytes upon in vivo augmentation of mouse natural killer activity. *J Immunol* 134:2799–810, 1985
29. Gidlund M, Orn A, Wigzell H, Senik A nd, Gresser I: Enhanced NK cell activity in mice injected with interferon and interferon inducers. *Nature* (Lond) 273:759–61, 1978
30. Fibbe WE, Van Damme J, Billiau A, Duinkerken N, Lurvink E, Ralph P, Altrock BW, Kaushansky K, Willemze R, Falkenburg JH: Human fibroblasts produce granulocyte-CSF, macrophage-CSF, and granulocyte-macrophage-CSF following stimulation by interleukin 1 and poly (rI), poly (rC). *Blood* 72:860–66, 1988
31. Akiyama Y, Stevenson GW, Schlick E, Matsushima K, Miller PJ, Stevenson HC: Differential ability of human blood monocyte subsets to release various cytokines. *J Leukocyte Biol* 37:519–30, 1985
32. DeMaeyer E, DeMaeyer-Guignard J: Macrophages as interferon producers and interferons as modulators of macrophage activity. In *Interferons and Other Regulatory Cytokines*. DeMayer E, DeMaeyer-Guignard J, Eds. New York, Wiley, 1988, p. 194–220