Administration of Silica Prevents Diabetes in BB-Rats

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SUMMARY

Administration of silica to young BB-rats almost completely prevented the development of spontaneous diabetes. Only 1 of 31 silica-treated rats developed hyperglycemia, whereas 9 of 31 in the untreated group did so. Since silica is highly specific in its action against macrophages, our observations indicate an important role of these cells in the pathogenesis of the disease.
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It has been reported that BB-rats spontaneously develop a diabetic syndrome characterized by mononuclear cell infiltration of pancreatic islets, islet cell antibody formation, and concomitant loss of islet B-cells.[1,2] An essential role of the immune system in disease pathogenesis is demonstrated by the finding that protection from hyperglycemia could be achieved by administration of antilymphocyte serum and cyclosporin A.[3,4]

Recent ultrastructural studies performed by us in the BB-rat clearly show an early phase of islet inflammation (single-cell insulitis) where single macrophages and lymphocytes invade the islet and concomitant destruction of islet B-cells is observed.[5,6]

We have therefore analyzed the role of macrophages in the pathogenesis of diabetes in the BB-rat by selective phagocyte inactivation experiments.

MATERIALS AND METHODS

Our BB-rat colony (BB/W/D) has been produced by a breeding stock obtained from Dr. A. A. Like, Department of Pathology at the University of Massachusetts Medical School. Sixty-two male and female BB/W/D from 10 litters were divided into age- and sex-matched subgroups (each litter subdivided into two matching groups). At the age of 60 and 65 days animals of one group received a total of 100 mg silica/kg body wt (particle size range 0.5–5 μm, Steinkohle-Bergbau-Verein, Essen, FRG) given i.p. and i.v. at two equal parts. Further injections were given i.p. at 70, 80, and 90 days of age.

Rats were checked regularly for weight increase, glycosuria, and ketonuria. Hyperglycemia was verified by blood glucose determination by the hexokinase method (Glukoquant, Boehringer Mannheim, FRG).

Histologic analysis of pancreatic sections was performed as described previously.[7] Peripheral blood lymphocytes were isolated by Ficoll-Hypaque (Pharmacia, Freiburg, FRG) density gradient centrifugation. The isolated leukocyte suspension contained 9–11% of polymorphonuclear cells as determined by Giemsa staining of cytospin preparations. Lymphocyte types were determined by indirect immunofluorescence using monoclonal antibodies W3/13 (reactive with thymocytes, T-cells, and polymorphonuclear cells), W3/25 (reactive with thymocytes and the T-cell subset containing helper cells), OX8 (reactive with thymocytes, the T-cell subset containing cytotoxic and suppressor cells, and with some NK cells) (all sera from Seralab, Wiesbaden, FRG). Affinity-purified, FITC-conjugated goal antimouse IgG (TAGO, Hamburg, FRG), which had been absorbed with rat erythrocytes, was used as the second antibody. Control slides first incubated with buffer or normal serum and then with FITC antimouse IgG had <2% positive immunofluorescent cells. All data are corrected for this background fluorescence. Statistical differences between the two groups were evaluated by the Wilcoxon-Mann-Whitney test.

RESULTS

The incidence of diabetes in silica-treated and untreated rats was followed up to the age of 180 days. In this period 1 of 31 (3%) silica-treated rats developed hyperglycemia compared with 9 of 31 (29%) untreated rats (Figure 1). The latter value is comparable with the frequency observed in our colony during these months (100 of 268 [37%]). The only diabetic animal in the silica-treated group developed hypergly-

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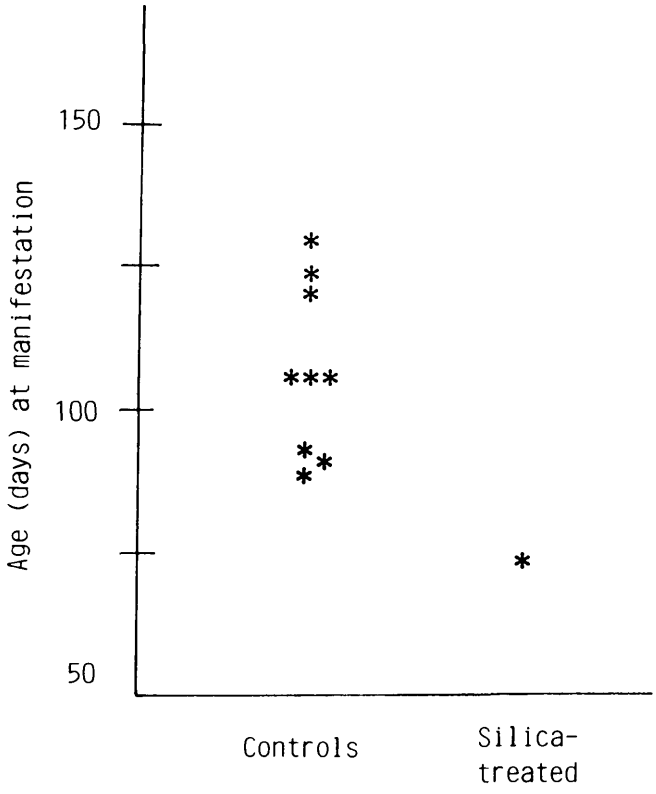

FIGURE 1. Incidence of diabetes in normal and silica-treated BB-rats. Of the control group of 31 animals, four male and five female rats developed diabetes. Of the silica group of 31 animals, one female rat developed diabetes at day 72.

cemia shortly after onset of silica treatment. The difference between the two groups is highly significant ($P < 0.005$).

Of each group 10 animals were killed for histologic examination at the age of 180 days (approximately 3 mo after peak incidence of insulitis and diabetes). Enlargement of parapancreatic lymph nodes was seen in the silica group. Diffuse granuloma tissue was found along the connective tissue associated with gut and pancreas. The nine nondiabetic rats did not show signs of insulitis. The one silica-treated, diabetic rat had fewer detectable islets, all with mononuclear cell infiltration. Two of the three diabetic rats in the control group also showed insulitis. The seven normoglycemic animals had normal pancreas histology. Silica-treated rats had a higher white blood cell count (Table 1). Silica treatment did not restore the T-cell deficiency of BB-rats. The number of T-lymphocytes (W3/13 antibody positive) and W3/25 (helper)- or OX8 (mostly cytotoxic/suppressor)-positive subsets in the peripheral blood did not differ significantly between the silica-treated and control groups. The spleen was enlarged but did not contain significantly more leukocytes than did control rats (Table 1).

Finally, a significant difference was seen in general growth; the body weight of silica-treated rats was persistently lower than in nondiabetic, untreated BB-rats (Table 1).

DISCUSSION

Our results show that silica treatment almost completely prevents spontaneous diabetes in BB-rats. The only case of diabetes in the silica-treated group occurred 12 days after start of treatment, indicating that silica administration in this rat may have been begun too late.

Many previous studies have shown silica to be highly specific in its action against macrophages; either destruction or chronic functional modification will occur.[8]

It cannot be decided at present whether the protective effect of silica administration is a direct consequence of macrophage depletion or modification or whether altered

TABLE 1
Comparison of silica-treated and untreated BB-rats at day 180

	Silica treatment*	Control*
Peripheral blood leukocytes (cells/μl) (N = 10)	6290 ± 840	5090 ± 470
W3/13 antibody-positive cells§ (cells/μl) (N = 10)	3630 ± 490	3860 ± 360
W3/25 positive cells‖ (% of total W3/13 positive cells) (N = 10)	12.4 ± 1.3	16.7 ± 1.5
OX8 positive cells¶ (% of total W3/13 positive cells) (N = 10)	39.7 ± 3.5	44.2 ± 1.2
Spleen weight (g wet weight) (N = 10)	0.82 ± 0.05†	0.62 ± 0.05
Spleen leukocytes (cells × 10^6) (N = 10)	165 ± 16	155 ± 11

Body weight# at the age of	Female (N = 16)	Male (N = 14)	Female (N = 10)	Male (N = 12)
60 days	171 ± 3	244 ± 12	171 ± 4	232 ± 10
120 days	223 ± 3‡	337 ± 10‡	249 ± 5	404 ± 7
160 days	237 ± 3‡	374 ± 12‡	269 ± 3	449 ± 7

*Data are mean values ± SEM, †$P < 0.01$, ‡$P < 0.001$; §T-lymphocytes and polymorphonuclear cells; ‖helper T-cells; ¶cytotoxic/suppressor plus some NK cells; #only nondiabetic animals have been considered.

macrophages act on T-lymphocytes in such a way that islet destruction is impaired.

Indeed it has been shown that a subpopulation of macrophages in BB-rats acts on T-lymphocytes and induces a depression in the proliferative response to T-dependent mitogens and of interleukin 2 production.[9] However, we did not observe normalization of T-cell depression after silica treatment with respect to the pronounced lack of helper T-cells in the peripheral blood of BB-rats. Another possibility is that macrophage from silica-treated animals has lost the capacity to present islet antigens to lymphocytes.

Macrophages function not only in an immunoregulatory capacity, but also as effector cells; for instance, silica treatment has been shown to block rejection of islet allografts.[10] We have provided evidence for an early phase of islet B-cell destruction (single-cell insulitis) where single macrophages and lymphocytes infiltrate the islet and attack islet B-cells.[5,6]

Furthermore, it has recently been shown that normal macrophages can spontaneously attack and destroy islet cells in vitro without prior stimulation by T-lymphocytes.[11] We therefore favor the idea that silica treatment of BB-rats prevents macrophages from infiltrating pancreatic islets.

An essential role for macrophage activity in autoimmune diabetes does not seem to be a unique property of BB-rats. We have found that the injection of silica particles to C57BL/Ks mice will completely inhibit the development of diabetes after multiple low-dose streptozocin treatment.[12] The protective effect of silica administration on diabetes development may qualify macrophages as a target for immunotherapeutic approaches.

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