Dimethyl Sulfoxide Modulation of Diabetes Onset in NOD Mice

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Dimethyl sulfoxide (DMSO), a hydroxyl radical scavenger, is known as an immunosuppressive agent and can reduce autoantibody levels in experimental autoimmune diseases. Because classic diabetogens damage the DNA and membrane of the β-cell by the generation of free radicals, the purpose of these investigations was to determine whether the intake of DMSO or its derivatives methylsulfonylmethane (MSM) and dimethylsulfide (DMS) could prevent the expression of autoimmune diabetes in the spontaneously diabetic NOD mouse. DMSO (2.5%), MSM (2.5%), and DMS (0.25%) were added to the drinking water of female NOD mice immediately after weaning. Control animals were maintained on regular drinking water. The presence of overt diabetes was monitored from the age of 2 mo by weekly urinary glucose testing until the animals either became overtly glucosuric or were >240 days of age. In contrast to what we expected, DMSO (2.5%) markedly increased the rate at which the animals expressed overt diabetes ($P < .0004$, log-rank test). MSM had no effect, whereas DMS reduced the incidence and rate of diabetes onset. When DMSO (2.5%) was administered to male NOD mice and control strains of mice (BALB/c and ICR), the control group did not develop glucosuria or insulitis, whereas DMSO increased the incidence of diabetes in the male NOD mice from 21 to 79%. In contrast, when DMSO was fed to female NOD mice on a purified AIN-76 diet, diabetes onset was reduced to 36%. We conclude that DMSO accelerates the uptake of dietary diabetogens into the β-cell of genetically susceptible animals (NOD mice). The protective effect of the purified diet in such animals may be due to a lack of putative diabetogens in purified diet, or alternatively, the diet itself contains factor(s) that protect the β-cell from autoimmune attack and/or destruction. *Diabetes* 38:194–97, 1989

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A role for environmental factors in the etiology of autoimmune diabetes mellitus in genetically susceptible humans and animals is gradually gaining credibility (1,2). Various studies have linked the toxic effects of agents such as alloxan (ALX), streptozocin (STZ), radiation, and inflammatory tissue damage on the β-cell into a common pathway that involves the generation of free radicals (3,4). For example, if animals are maintained in an antioxidant-depleted state, they demonstrate increased diabetogenic susceptibility to nondiabetogenic doses of these compounds, whereas the simultaneous administration of agents that control free radical levels or their effects (e.g., vitamin E, nicotinamide) provides protection against such action (5,6). Dimethyl sulfoxide (DMSO) is another compound that can attenuate the effects of ALX on the β-cell (7). DMSO may be more effective than catalase or superoxide dismutase because it is a small, highly permeable molecule that has the ability to reach intracellular locations. DMSO also functions as an immunosuppressant and can reduce the autoantibody levels in experimental autoimmune diseases (8–10). Thus, the combined potential of DMSO as an immunosuppressive agent and a free-radical scavenger suggests a possible role for this compound in the prevention of autoimmune diabetes. We investigated the effect of DMSO and its in vivo metabolites, methylsulfonylmethane (MSM) and dimethylsulfide (DMS) on the development of diabetes in the NOD mouse. In this model, insulitis develops in essentially all of these inbred animals, although overt hyperglycemia develops in only 70% of females and 20% of males.

MATERIALS AND METHODS

Control strains of mice (BALB/c, ICR) not susceptible to diabetes were obtained commercially, housed three per cage, and allowed free access to pelleted Ralston Purina Chow. NOD mice were inbred and maintained under pathogen-free conditions. After weaning, the NOD mice were housed two to three per cage. At 2 mo of age, all animals were tested weekly for the presence of glucosuria with Tes-

Tape (Lilly, Indianapolis, IN) and were classified as overtly diabetic on the basis of Tes-Tape values >2+. When the animals were ≥240 days old, or when they became overtly diabetic, they were decapitated, and blood was collected to measure plasma glucose (Autoanalyzer, Technicon, Tarrytown, NY).

Pancreases from nondiabetic animals were stored in formalin before histologic examination. Three sections (4 μm) from each animal were stained with hematoxylin and eosin and scored for lymphocytic infiltration. Histology scores were 0, no inflammatory cells observed in the islet; 1, infiltrating cells observed in periductal and/or perivascular locations; 2, relatively small numbers of islet-associated cells at the islet periphery (early-stage insulitis); 3, moderate to severe inflammation of the islet; and 4, infiltrating mononuclear cells permeate the islet tissue, and evidence of β-cell necrosis is seen.

DMSO (2.5%, 99.9% pure, Sigma St. Louis, MO), MSM (2.5%, Sigma), and DMS (0.25%, Calbiochem, La Jolla, CA) were added to the drinking water of the mice immediately after weaning. The dose of DMSO we used (10 g · kg $^{-1}$ · day $^{-1}$) exceeded that used by Pestronk and Drachman (8), who effectively reduced serum levels of IgG in mice with daily injections of 4.4 g/kg i.p. DMSO. Although the oral administration of DMSO (2.5%) did not affect spleen or body weight, it was reported that DMSO increased the daily fluid intake from 5.5 to 10 ml · day $^{-1}$ · mouse $^{-1}$ (10). In one study, sucrose (10%; Mallinckrodt, Paris, KY) was added to the drinking water of male NOD mice to determine whether an increase in β-cell activity could affect diabetes onset. Diabetes incidence was compared with a group of male or female NOD mice (referred to hereafter as *colony mice*) maintained on regular tap water and randomly selected from the same generation as those in the other studies described herein.

The purified AIN-76 diet was purchased from Teklad (Madison, WI). Its composition is given in Table 1, and it was provided ad libitum to the mice.

The survival distributions were estimated with the product-limit method (11), with the results compared by use of the log-rank test (12; Mantel-Haenszel test for survivorship data). The test compares the survival duration of treated animals with appropriate control animals by χ^2-analysis. This method has several distinct advantages over the more traditional analysis of variance (ANOVA) of the survival time: *1*) the method is nonparametric and unlike ANOVA does not require the usual assumption of normality of the data; *2*) all of the data are used, instead of a single chosen time; and 3) even partial data can be included in the analysis (i.e., if an observation is lost during the experiment, the log-rank test is able to use the observation data up to that point). The statistical software used was BMDP, PI L program (13).

TABLE 1
Composition of purified AIN-76 diet

Ingredients	g/kg
Casein, high protein	200.0
DL-Methionine	3.0
Sucrose	499.97
Cornstarch	150.0
Corn oil	50.0
Cellulose	50.0
Mineral mix, AIN-76	35.0
Vitamin mix, AIN-76A	10.0
Choline bitartrate	2.0
Ethoxyquin	0.03

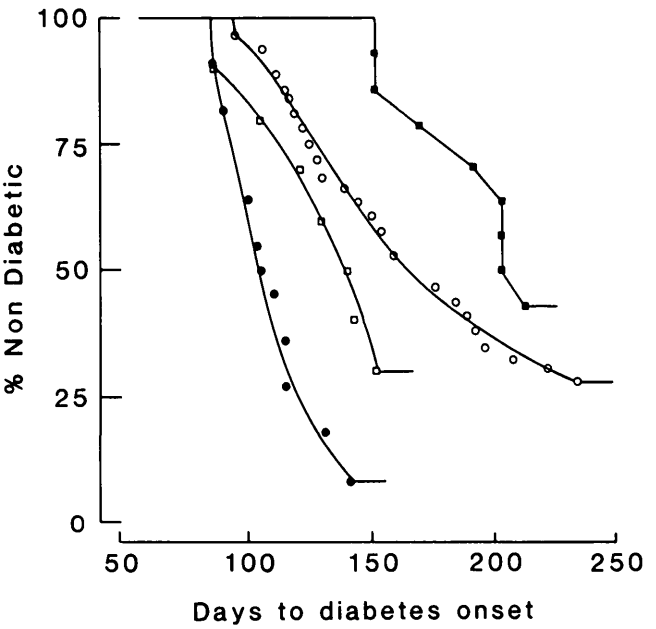

FIG. 1. Percentage nondiabetic female NOD mice administered dimethyl sulfoxide (●, 2.5%; *n* = 11), methylsulfonylmethane (□, 2.5%; *n* = 10) and dimethylsulfide (■, 0.25%; *n* = 14) in drinking water from time of weaning. Associated probabilities are relative to colony mice (○; *n* = 36) that received regular tap water and were randomly selected from same generation as those used in our study.

RESULTS

Effect of DMSO, MSM, and DMS on incidence of diabetes occurrence in female NOD mice. The effect of DMSO (*n* = 11), MSM (*n* = 11), and DMS (*n* = 14) on the incidence of diabetes in female NOD mice is shown in Fig. 1. In contrast to what we expected, DMSO accelerated the rate at which the mice became diabetic ($P < .004$ vs. colony) and increased the percentage of animals expressing overt diabetes (72% colony vs. 91% DMSO). There was no significant effect of MSM on diabetes incidence, but DMS delayed diabetes onset (1st animal diabetic on DMSO, 85 days; on DMS, 151 days) and reduced the number of animals expressing the disease (72% colony vs. 57% DMS). The median quantity of the survival distribution was 204 days for DMS, 159 days for colony, 140 days for MSM, and 106 days for the DMSO animals.

Effect of DMSO on incidence of diabetes in male NOD mice and control mice. Because of the unexpected nature of these findings, additional studies were conducted in two non-diabetes-prone strains of mice (BALB/c and ICR) as well as in male NOD mice. BALB/c (*n* = 12), ICR (*n* = 7), and male NOD (*n* = 14) mice were given DMSO (2.5%) in their drinking water immediately after weaning. Animals in the control strain did not demonstrate any glucosuria and were killed when they were 200 days of age. Histologic examination of the pancreas revealed no lymphocytic islet infiltrates in any of the BALB/c or ICR mice.

The addition of DMSO to the drinking water of the male NOD mice paradoxically increased the incidence of diabetes to 79% (Fig. 2). There was no effect of the sucrose solution on diabetes onset, with 30% of the animals expressing glucosuria. The incidence of diabetes in this particular generation of males was 21%.

Effect of DMSO on insulitis and incidence of diabetes in female NOD mice fed purified diet. Studies in the spontaneously diabetic BB rat have demonstrated that feeding a purified AIN-76 diet instead of a standard laboratory chow markedly reduced the severity and incidence of insulitis and completely prevented diabetes onset in the genetically susceptible animals (14). To determine whether feeding a purified diet to female NOD mice drinking DMSO is effective for reducing insulitis and diabetes onset compared with mice eating the regular laboratory chow was the purpose of our study. To this end, female NOD mice were administered DMSO (2.5%) in their drinking water immediately after weaning. One group of animals (n = 15) was placed on the regular laboratory chow, and a second group was fed the purified AIN-76 diet (n = 14). Food and water were provided ad libitum.

As in the first experiment, DMSO markedly increased the rate at which the animals became diabetic when fed the laboratory chow (Fig. 3). However, in this study the percentage of mice expressing the disease was similar to that of the colony mice. The percentage and rate of incidence of overt diabetes was markedly reduced to 36% in animals fed the purified diet. The degree of insulitis in the nondiabetic DMSO animals fed the purified diet (2.3 ± 0.5, mean ± SE; n = 4) was not significantly different from those fed the chow diet (2.5 ± 0.4; n = 5) or maintained on tap water (2.3 ± 0.2).

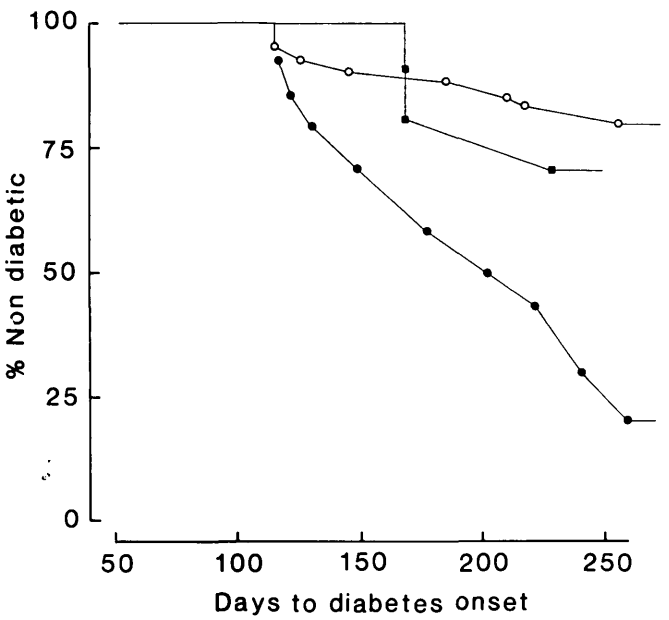

FIG. 2. Percentage nondiabetic male NOD mice administered dimethyl sulfoxide (●, 2.5%; n = 14) or sucrose (■, 10%; n = 10) in drinking water from time of weaning. Comparisons are relative to colony mice (○; n = 38) that received regular tap water and were randomly selected from same generation as those used in our study.

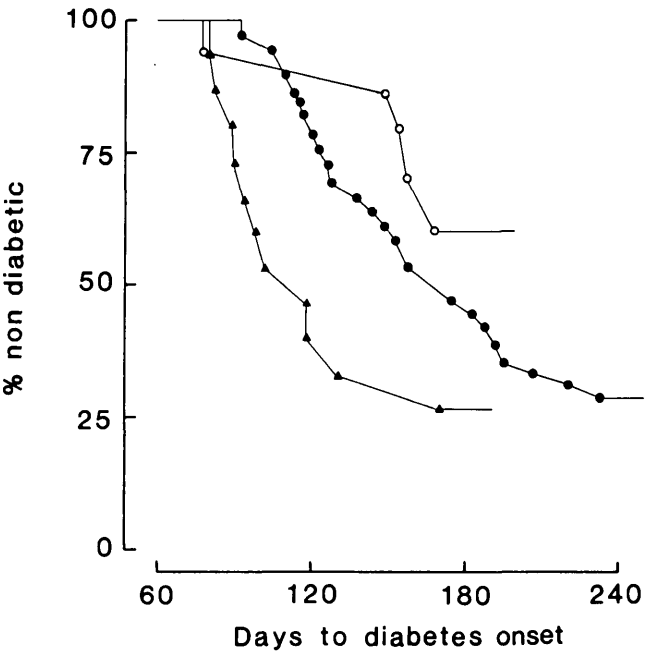

FIG. 3. Percentage nondiabetic female NOD mice administered dimethyl sulfoxide (2.5%) in drinking water and fed purified AIN-76 diet (○; n = 14) or regular chow (▲; n = 15). At time of weaning, pups from each litter were equally divided into 2 groups. Percentage of survivorship of female colony (●; n = 36) is shown for comparison.

DISCUSSION

Our study indicates that DMSO and its derivative DMS are able to affect the onset of diabetes in the NOD mouse. DMS is a potent antioxidant (15), and the delay in diabetes onset as well as the reduced incidence of animals expressing the disease are consistent with this effect. However, even though DMSO is also an antioxidant, the mechanism by which DMSO accelerates diabetes onset has never been observed or established. DMSO could increase the absorption of an as yet unidentified diabetogen contained in the regular diet into the β-cell, or it could exert a direct effect on the β-cell itself. In high concentrations (0.7 M), DMSO has been shown in vitro to perturb the membrane of the rat β-cell and cause a substantial monophasic release of insulin (16,17). Human islets perfused with low concentrations of DMSO (32 mM) also respond to DMSO with a pronounced sustained release of insulin (18). However, because the incidence of diabetes in male NOD mice drinking sucrose-containing water was not significantly different from that of the colony mice, it is unlikely that an increase in β-cell activity per se contributes to diabetes onset.

DMSO could potentially react with hydroxide generated in vivo and accelerate the production of superoxide radicals (19). If this reaction were to occur in the vicinity of the β-cell membrane, it could lead to lipid peroxidation and other radical-induced membrane damage (20). Another diabetogenic effect of DMSO is its potential for inducing single-strand breaks in the DNA of various organs of mice (21). STZ and ALX are also known to damage the DNA of the β-cell, which ultimately contributes to immune attack against the β-cells (3).

It is known that DMSO and its derivatives are found in many fruits, grains, and vegetables and that crop plants

absorb DMSO via the roots and foliage (22). Most of the biotransformations occur in the foliage of the plants, where DMSO is incorporated into plant protein and the sulfur-containing amino acids. DMSO can be oxidized to MSM and reduced to the volatile DMS by plant tissues (23). DMSO is thus a normal constituent of the food chain and is present as a codiabetogen. Whether the food chain contains sufficient levels of this material to influence the expression of diabetes in genetically susceptible individuals is speculative but remains an intriguing possibility. DMSO nevertheless represents an important dietary constituent and, with respect to its clinical use (15), may be a factor in the etiology of insulin-dependent diabetes.

Further support for the view that environmental codiabetogens may be important factors in the incidence of diabetes comes from the recent epidemiological studies of Krolewski et al. (2). Despite the fact that the gene pool that predisposes subjects to diabetes has remained relatively constant, the incidence of diabetes in young subjects has almost tripled in the past three decades. Much of the increase has been measured in the 5- to 14-yr-old age group. These data suggest that the rising incidence of insulitis is due to a change in exposure to unidentified environmental agents. Thus, the effectiveness of the purified diet in reducing the incidence and rate at which the animals became diabetic may have been due to the absence of an unidentified diabetogen. Alternatively, the AIN-76 diet may contain protective factors that modulate the immune response and autoantibody production.

DMSO is certainly a candidate as an environmental codiabetogen and represents another tool in the investigation of factors that modulate diabetes onset.

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