PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **183,** 227–230 (1986)

Effects of Oral Dimethyl Sulfoxide and Dimethyl Sulfone on Murine Autoimmune Lymphoproliferative Disease[1] (42409)

JANE I. MORTON AND BENJAMIN V. SIEGEL

Division of Arthritis and Rheumatic Diseases and Department of Pathology, Oregon Health Sciences University, Portland, Oregon 97201

Abstract. The results from several studies examining the effects of DMSO on autoimmune phenomena have been inconclusive, possibly because of differences in experimental models, treatment regimens and doses employed. In the present investigation, autoimmune strain MRL/*lpr*, C3H/*lpr*, and male BXSB mice were placed on a continuous treatment regimen with 3% DMSO or 3% $DMSO_2$ in the drinking water, *ad libitum*, commencing at 1 to 2 months of age, before spontaneous disease development could be detected. This represented doses of 8–10 g/kg/day of DMSO and 6–8 g/kg/day of $DMSO_2$. Both compounds were observed to extend the mean life span of MRL/*lpr* mice from $5\frac{1}{2}$ months to over 10 months of age. All strains showed decreased antinuclear antibody responses and significant diminution of lymphadenopathy, splenomegaly, and anemia development. Serum IgG levels and spleen IgM antibody plaque formation, however, did not differ from control values. There was no indication of involvement of systemic immunosuppressive or antiproliferative effects, and treated animals were observed to remain healthy and vigorous with no signs of toxicity. These results demonstrate that high doses of both DMSO and its major *in vivo* metabolite, $DMSO_2$, provide significant protection against the development of murine autoimmune lymphoproliferative disease. Possible mechanisms of protection are discussed.

It has been difficult to draw definitive conclusions regarding the effects of dimethyl sulfoxide (DMSO) treatment on the development of autoimmune phenomena, possibly because of differences in species employed, experimental models of disease selected, and/or the treatment protocols. Previous studies from this laboratory (1) have demonstrated that ip injection of 0.10 ml of 50% DMSO, three times a week for 20 weeks, had little effect on the spontaneous development of antinuclear antibodies (ANA) or on abnormal lymphoproliferation in autoimmune strain NZB, MRL/*lpr*, or male BXSB mice. However, where rats have been induced experimentally to develop myasthenia gravis (2), collagen arthritis (3), or autoimmune thyroiditis (4), autoantibody titers were found to be decreased with DMSO injection.

One possible explanation for the observed differences may be that adequate prophylactic levels of DMSO were not achieved in the case of the autoimmune mouse strains. To explore this possibility, autoimmune-prone mice were treated with DMSO as a 3% solution in the drinking water, *ad libitum*. The experiments were also extended to include 3% drinking water solutions of dimethyl sulfone ($DMSO_2$), the major *in vivo* metabolite of DMSO, with separate groups of mice. The dosages achieved in this manner were 8–10 g/kg/day of DMSO and 6–8 g/kg/day of $DMSO_2$. Treatments were started at 1 to 2 months of age, before disease was evident, and animals were examined for disease development 4 months or more later. All strains showed significant decreases in the development of anemia, lymphadenopathy, and antinuclear antibodies; in addition, the MRL/*lpr* mice studied over the long term manifested marked life span extension.

Materials and Methods. Colonies of MRL/*lpr*, C3H/*lpr* and BXSB mice were originally obtained from Dr. E. Murphy and J. Roths, The Jackson Laboratory (Bar Harbor, Maine) and have been continuously maintained in this laboratory. Mice were housed in shoeboxes on hardwood bedding and fed Purina Laboratory Chow. Three percent solutions of DMSO (Merck) and of $DMSO_2$ (Showa Chemical Co.,

[1] This study was supported by the Gerlinger Research Fund, the Ronald J. Purer Foundation, the DMSO Foundation, and the Oregon Arthritis Foundation.

0037-9727/86 $1.50

Osaka, Japan) were administered *ad libitum* as drinking water. These solutions, kindly provided by Dr. S. W. Jacob, Oregon Health Sciences University, showed only one peak on chromatographic analysis. Plasma antinuclear antibodies were analyzed employing an indirect immunofluorescence assay with chicken erythrocyte nuclei as substrate as previously described (5). Responses were graded on a scale of 0 to 4, with 3 and 4 considered strongly positive. Serum IgG was measured by radial immunodiffusion utilizing a quantitative immunodiffusion kit (Miles Laboratory, Elkhart, Ind.). Direct antibody plaque formation was measured (6) using spleen cells from C3H/*lpr* mice that had been injected ip with 5×10^8 washed sheep erythrocytes (SRBC) 5 days previously.

Results and Discussion. MRL/*lpr* strain mice have an average life span of approximately $5\frac{1}{2}$ months (7). As noted in Fig. 1, survivals were extended beyond 10 months of age in the case of mice ingesting 3% solutions of DMSO or $DMSO_2$ continuously, starting at 5 to 7 weeks of age. DMSO treatment also resulted in a reduced incidence of strong ANA positivity and significant decreases in spleen, thymus, and lymph node weights (Table I). Eighteen-week-old DMSO-treated MRL/*lpr* mice showed significantly diminished lymphoid organ enlargement compared to water-drinking controls but were clearly still abnormal. On the other hand, lymphadenopathy was virtually absent in the 43-week-old group of animals and only one-sixth showed strong ANA reactivity (Table I). The reason for the occurrence of decreased disease in older compared to younger MRL/*lpr* mice treated with DMSO is not known, but could conceivably be associated with a diminution in lymphoproliferative activity with age. It should be noted that lymphoproliferation induced by the *lpr* gene does not fit the generally accepted criteria of malignancy such as transplantability (8). In this connection, the enlarged nodes have been observed to consist of accumulations of resting cells resulting from migration into the area of cells which have undergone proliferation at other sites (9).

C3H/*lpr* strain mice, like the MRL/*lpr*, are congenic for the somatic recessive lymphoproliferation (*lpr*) gene and develop similar lymphadenopathy. However, due to different interacting background genes, development of antinuclear antibodies and immune complex renal disease is less pronounced than in the MRL/*lpr* (8) and survivals are longer, often up to 1 year of age. In Fig. 2A, the massive lymphadenopathy obtaining in a representative untreated 6-month-old C3H/*lpr* mouse is illustrated by way of comparison to a similar animal which had been treated with 3% DMSO from 2 months of age (Fig. 2B). Treated mice appeared healthy and vigorous with little or no external signs of lymph node enlargement and with no evidence of drug toxicity. Similar effects were noted with 3% $DMSO_2$ administration. In addition to reduc-

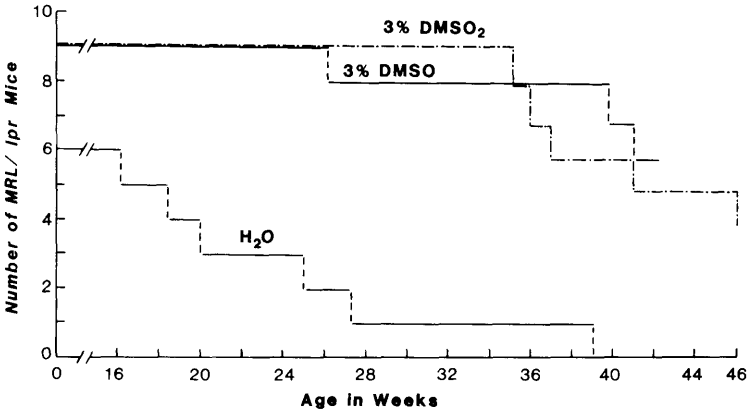

FIG. 1. Numbers of male MRL/*lpr* mice alive at given ages are shown for animals drinking tap water, 3% DMSO, or 3% $DMSO_2$, *ad libitum*. DMSO and $DMSO_2$ treatments were started at 35 to 45 days of age.

TABLE I. EFFECT OF ORAL DMSO ON IMMUNE DISEASE IN MALE MRL/lpr MICE

Parameter	Water controls (18 weeks old)	3% DMSO[a] (18 weeks old)	3% DMSO (43 weeks old)
Survivals	10/17	8/8	6/9
Strong ANA response[d]	10/10	3/8	1/6
Hematocrit (%)	47.7 ± 1.0	47.9 ± 0.46	47.8 ± 0.91
Spleen wt (mg)	545.9 ± 51.6	201.8 ± 24.9[b]	243.2 ± 44.5[c]
Thymus wt (mg)	366.1 ± 42.8	167.0 ± 23.6[c]	88.9 ± 28.7[b]
Node wt (mg)	3680 ± 330	996 ± 350[b]	102 ± 43[b]

[a] Mice terminated at 18 weeks of age were started on 3% DMSO v/v in the drinking water at 24 days of age. Mice terminated at 43 weeks of age were started on 3% DMSO at 35 to 45 days of age. Data are expressed as means ± SEM.
[b] Different from water control at $P < 0.001$.
[c] Different from water control at $P < 0.005$.
[d] Serum antinuclear antibody (ANA) responses were evaluated by indirect immunofluorescence assay (5).

ing lymph node mass, DMSO significantly diminished anemia and decreased the incidence of strong ANA reactivity from 46 to 17% (Table II).

Male BXSB mice, which develop a lupus-like immune complex renal disease as the result of an autoimmune accelerator on the Y chromosome (7), also showed diminished disease development when drinking 3% DMSO (Table II). Here, splenomegaly was significantly reduced, anemia was prevented, and the incidence of strong ANA positivity decreased from 64 to 11%.

The mechanisms for the protective effects of DMSO and $DMSO_2$ on the spontaneous development of autoimmune-lymphoproliferative disease in the different mouse models have not been established. We have found DMSO and $DMSO_2$ to be virtually identical in their ability to diminish the severity of these disorders, including the rheumatoid arthritis-like joint lesions characteristic of the MRL/*lpr* strain (10). It is conceivable that $DMSO_2$, which is the major *in vivo* metabolite of DMSO (11), may be the biologically effective molecule in these reactions. Lending support to this view is the observation that BALB/c mice chronically ingesting 3% DMSO manifested approximately equivalent serum levels of DMSO and $DMSO_2$ (200–400 μg/ml) (Layman and Morton, unpublished results).

While autoimmune-lymphoproliferative disease was diminished with continuous ingestion of 3% DMSO and $DMSO_2$, the primary IgM response of DMSO-treated C3H/*lpr* mice following SRBC immunization was not altered, and serum IgG levels in C3H/*lpr* and BXSB male mice remained unchanged (Table II). In other studies from this laboratory (12), similar treatment of nonautoimmune strain BALB/c

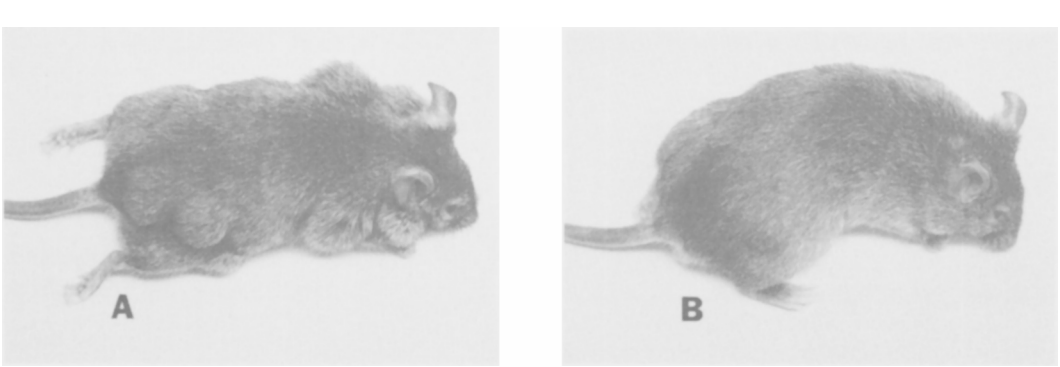

FIG. 2. The gross appearance of representative 6-month-old male C3H/*lpr* mice drinking tap water (A) and drinking 3% DMSO (B) continuously from the age of 2 months.

TABLE II. EFFECT OF ORAL DMSO ON IMMUNE DISEASE IN MALE BXSB AND C3H/1pr MICE[a]

Parameter	BXSB		C3H/1pr	
	H_2O	DMSO	H_2O	DMSO
Hematocrit (%)	39.1 ± 0.72	45.3 ± 0.83[b]	41.5 ± 0.62	44.1 ± 0.63[b]
Thymus wt (mg)	64.9 ± 12.0	34.6 ± 5.9[b]	71.2 ± 9.5	38.4 ± 4.6[b]
Spleen wt (mg)	530.2 ± 101	269.0 ± 38.3[b]	526.4 ± 29.3	316.9 ± 19.6[b]
Node wt (mg)	41.3 ± 11.0	35.0 ± 6.0	4500.0 ± 170.0	1830.0 ± 220.0[b]
Primary antibody (IgM PFC/spleen)	—	—	45,851 ± 11,300	46,081 ± 8,200[c]
Serum IgG (mg%)	739 ± 80.4	589 ± 33.8[c]	633 ± 65.7	570 ± 79.1[c]
Strong ANA response[d]	9/14 (64%)	2/18 (11%)	5/11 (46%)	2/12 (17%)

[a] Male BXSB and C3H/1pr mice were started on 3% DMSO v/v in drinking water at 2 months of age and assays performed 4 months later.

[b] Difference between mice on DMSO and water significant, $P < 0.05$.

[c] Difference between mice on DMSO and water not significant, $P > 0.05$.

[d] Serum antinuclear antibody (ANA) responses were measured by an indirect immunofluorescence assay using chicken erythrocyte nuclei as substrate (5).

mice was without significant effect on lymphoid organ, liver, or body weights; also T-dependent and T-independent spleen antibody plaque-forming cell responses manifested little or no differences from those of water-drinking controls. Systemic immunosuppressive and/or antiproliferative effects would not appear, thus, to be the mechanisms by which DMSO and $DMSO_2$ provide protection. Possible changes in such factors as antibody subclasses produced, immune complex formation and clearance, and serum complement levels could be implicated. Conceivably too, alterations in the activity of immunoregulatory molecules, such as prostaglandins and lymphokines, might contribute to the protective action of these compounds. Clarification of the role of one or more of these factors in disease development and its modulation by DMSO or $DMSO_2$ treatment should be obtained from further investigations.

1. Morton JI, Siegel BV, Weaver WJ, Bristol T, Jacob SW. The effect of chronic DMSO administration on the spontaneous development of autoimmune disease in NZB, BXSB and MRL/lpr strain mice. Ann NY Acad Sci **411**:344–346, 1983.
2. Pestronk A, Drachman DB. Dimethyl sulphoxide reduces anti-receptor antibody titers in experimental myasthenia gravis. Nature (London) **288**:733–734, 1980.
3. Trentham DE, Rowland D. Dimethyl sulfoxide does not suppress the clinical manifestations of collagen arthritis. J Rheumatol **10**:114–116, 1983.
4. Weetman AP, Rennie DP, McGregor AM, Hall R. Support for an immunosuppressive action of dimethylsulfoxide. Clin Immunol Immunopathol **25**:53–58, 1982.
5. Siegel BV, Brown M, Morton JI. Detection of antinuclear antibodies in NZB and other mouse strains. Immunology **22**:457–463, 1972.
6. Cunningham AJ, Szenberg A. Further improvements in the plaque technique for detecting single antibody forming cells. Immunology **14**:599–600, 1968.
7. Murphy ED, Roths JB. Autoimmunity and lymphoproliferation: Induction by a mutant gene lpr and acceleration by a male associated factor in strain BXSB mice. In: Rose N, Bigazzi P, Warner NL, eds. Genetic Control of Autoimmune Disease. New York, Elsevier/North-Holland, pp207–221, 1978.
8. Theofilopoulos AN, Dixon FJ. Murine models of SLE. Adv Immunol **37**:269–390, 1985.
9. Raveche ES, Steinberg AD, DeFranco AL, Tjio J.-H. Cell cycle analysis of lymphocyte activation in normal and autoimmune strains of mice. J Immunol **129**: 1219–1226, 1982.
10. Moore RD, Morton JI. Diminished inflammatory joint disease in MRL/lpr mice ingesting dimethylsulfoxide (DMSO) or methylsulfonylmethane (MSM). Fed Proc **44**:530, 1985.
11. Gerhards E, Gibian H. The metabolism of dimethyl sulfoxide and its metabolic effects in man and animals. Ann NY Acad Sci **141**:65–76, 1967.
12. Mosesian J, Jensen P, Morton JI. Humoral immunity in mice treated with dimethylsulfone and dimethylsulfoxide. Proc Oreg Acad Sci **20**:6, 1984.

Received April 14, 1986. P.S.E.B.M. 1986, Vol. 183.
Accepted July 14, 1986.