EFFECT OF SPERGUALIN IN AUTOIMMUNE DISEASE MICE

KYUICHI NEMOTO, MICHIKO HAYASHI, JUNPEI ITO, FUMINORI ABE,
TOMOHISA TAKITA, TERUYA NAKAMURA[†],
TOMIO TAKEUCHI[††] and HAMAO UMEZAWA[††,†††]

Research Laboratories, Pharmaceuticals Group, Nippon Kayaku Co., Ltd.,
3-31-12 Shimo, Kita-ku, Tokyo 115, Japan
[†]Central Research Laboratories, Takara Shuzo Co., Ltd.,
3-4-1 Seta, Ohtsu-shi, Shiga 520-21, Japan
[††]Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

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We investigated the effect of spergualin (SGL) upon the development of spontaneous systemic lupus erythematosus-like lesions in male MRL/MpJ-lpr/lpr mice. SGL was administered ip at doses of 2.5, 5 and 10 mg/kg to two groups of mice. One group received SGL prophylactically from 7 to 21 weeks of age. The other group received SGL curatively from 13 to 27 weeks of age. The occurrence of lupus lesions was characterized by enlarged lymphoid organs, high anti-DNA titer and blood urea nitrogen, and severe glomerular nephritis. In both groups these characteristics were significantly suppressed by SGL in a dose-dependent manner. This inhibitory activity was greatest at a dose of 10 mg/kg. These findings suggest that SGL has prophylactic and curative effects against lupus lesions in autoimmune disease in mice.

Spergualin (SGL) is an antibiotic that was discovered in culture filtrate of *Bacillus laterosporus* BMG162-aF2[1] and strongly suppresses various immune responses[2,3]. Recently, we showed that this compound suppressed the experimental allergic encephalomyelitis in guinea pigs immunized with isogenic spinal cord[4]. In the present study, the effect of SGL on systemic lupus erythematosus (SLE)-like lesions developing spontaneously in MRL/MpJ-lpr/lpr (MRL/l) mice was examined.

Materials and Methods

Animals

Male MRL/l and MRL/MpJ-+/+ (MRL/+) mice were purchased from Jackson Laboratories, Bar Harbor, U.S.A.

Treatment

SGL was supplied by Takara Shuzo Co., Ltd., dissolved in saline, sterilized by passing through a 0.22-μm filter and stored at $-20°C$ until use. In the SGL-treated mice, the drug was administered ip daily at doses of 2.5, 5 and 10 mg/kg for 4 weeks. For mice in Expt I (prophylactic group), SGL was then administered from week 7 through 21. For mice in Expt II (curative group), SGL was administered from week 13 through 27. During the intervening weeks no SGL was given to either group. A set of control mice received saline was included in each experimental group. The mice were sacrificed 1~2 days after completion of the SGL administration, and lymph nodes, spleens, blood and kidneys were removed and examined as follows:

Peripheral Blood Cell Count

Peripheral red blood cell (RBC) and white blood cell (WBC) counts were performed using a

[†††] Deceased.

standard hemocytometer. Hematocrit (Ht) was performed with microhematocrit tubes. WBC differentials were determined by enumerating 100 or 200 cells in May-Grüenwald-Giemsa-stained smears.

Measurement of Anti-DNA Antibody by Enzyme-linked Immunosorbent Assay (ELISA)

ELISA was carried out by the modified methods of EATON *et al.*[5] and GODFREY *et al.*[6]. An aliquot (0.3 ml) of 0.05% aq solution of poly-L-lysine was incubated in a Nunc ELISA plate for 1.5 hours at room temp. The plates were washed using phosphate buffered saline with 0.1% gelatin (PBS-G) as the washing or diluting buffer, and 0.2 ml of calf thymus DNA (5 μg/ml PBS-G; Sigma, St. Louis, U.S.A.) was added. After incubating overnight at 37°C, any free sites were blocked by incubating with 0.2% bovine serum albumine for 1 hour at 37°C. A 0.2-ml volume of a 1/100 dilution of each mouse serum was then added to the plates and incubated for 1.5 hours at 37°C. The plates were washed, and 0.2 ml of a 1/1,000 dilution of goat anti-mouse IgG serum (Cappel, Cochranville, U.S.A.) was added and incubated for 1.5 hours at 37°C. After washing, a 1/1,000 dilution (0.2 ml) of alkaline phosphatase-conjugated rabbit anti-goat IgG serum (Cappel) was added and incubated 1.5 hours at 37°C. Finally, 0.2 ml of *p*-nitrophenyl phosphate (Sigma) was added at 2 mg/ml in 10% diethanolamine buffer, pH 9.8, and after 1 hour incubation at room temp the absorbance of *p*-nitrophenyl was measured at 405 nm.

Blood Urea Nitrogen (BUN)

BUN was estimated using Rapid Blood Analyzer Super (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan).

Histology

Kidneys were fixed in 10% buffered formaline and sectioned. The sections were stained with hematoxylin-eosin, periodic acid shiff, Masson and fibrin-staining methods for histopathological examinations. Twenty-five glomeruli were observed per section and the degree of glomerular damage was indicated by a pathological score based on the severity of change where 0=no, 1=minimal, 2=slight, 3=moderate and 4=severe.

Statistical Analysis

Data were analyzed by Student's t-test.

Results

MRL/l mice spontaneously develop massive generalized lymphadenopathy with proliferation of nonmalignant lymphocytes. We measured the weight of the lymphoid organs to evaluate the effect of SGL on the enlargement of lymph nodes and spleens. As shown in Table 1, SGL inhibited enlargement of these organs in both groups of mice. This effect was greatest at the maximum dose of 10 mg/kg.

Compared to MRL/+ mice, the untreated MRL/l mice had a markedly higher WBC count due to increases in lymphocytes and granulocytes (Table 2). SGL administration significantly lowered the WBC count in both groups of mice. These results were due to decreases in both lymphocyte and granulocyte counts. In contrast, no decreases were observed in the RBC number and Ht in the groups receiving SGL.

MRL/l mice are also characterized by the development of antibody to DNA and by glomerulonephritis. As shown in Table 3, high anti-DNA titers and BUN values were associated with a severe glomerulonephritis in the control mice. However, SGL at doses of 5 and 10 mg/kg significantly suppressed these three parameters. At the time of sacrifice (27 weeks old mice), 4 out of 10 control mice had died due to severe glomerulonephritis. The SGL-treated mice receiving at 5 and 10 mg/kg did

Table 1. Effect of SGL on enlargement of lymphoid organs in MRL/l mice.

Expt	SGL (mg/kg)	n	Lymph node (g)		Spleen (g)
			Mesenteric	Axillary	
I	0	10	1.47±0.57	0.85±0.22	0.57±0.08
	2.5	10	0.83±0.41	0.74±0.22	0.31±0.07**
	5	10	0.50±0.23**	0.42±0.17**	0.19±0.05**
	10	9	0.10±0.07**	0.11±0.08**	0.10±0.02**
II	0	6	1.22±0.23	0.79±0.29	0.70±0.14
	2.5	8	0.90±0.42	1.02±0.43	0.49±0.26
	5	9	0.60±0.42**	0.51±0.27*	0.23±0.05**
	10	7	0.18±0.05**	0.15±0.05**	0.13±0.04**

Data are shown as mean±SD.
* $P<0.05$. ** $P<0.01$.

Table 2. Peripheral blood cell counts in SGL-treated MRL/l mice.

Expt	SGL (mg/kg)	RBC ($\times 10^4/\mu l$)	Ht (%)	WBC ($\times 10^2/\mu l$)	$\times 10^2/\mu l$	
					Lymphocyte	Granulocyte
I	0	882±48	45±3	2,127±260	1,333±168	752±98
	2.5	849±58	46±3	263±28**	158±16**	100±13**
	5	924±26	48±3	514±187**	325±122**	187±67**
	10	894±32	48±2	287±95**	187±68**	100±34**
II	0	849±73	45±1	1,259±452	751±375	288±122
	2.5	856±80	45±3	462±150*	292±94	164±61
	5	898±34	43±2	516±130*	298±61	216±71
	10	881±42	44±2	464±116*	249±88	206±59

Data are shown as mean±SE except that Ht as mean±SD.
* $P<0.05$. ** $P<0.01$.

Table 3. Therapy of lupus nephritis in MRL/l mice by SGL.

Expt	SGL (mg/kg)	Anti-DNA titer† (A_{405} nm)	BUN (mg/dl)	Degree of glomerular damage (score)
I	0	0.48±0.24	38.7±9.0	1.8±0.5
	2.5	0.43±0.31	35.8±8.6	1.6±0.2
	5	0.18±0.06*	21.1±2.5*	0.9±0.3*
	10	0.12±0.06*	16.2±2.3*	0.3±0.2*
II	0	0.58±0.30	43.0±9.7	2.7±0.5
	2.5	0.49±0.27	38.8±7.9	2.1±0.5*
	5	0.26±0.09*	28.0±5.5*	1.6±0.5*
	10	0.17±0.07*	25.9±8.0*	0.7±0.6*

† MRL/+ mice of the same age did not have detectable anti-DNA titer ($A_{405} \leqq 0.1$) in 1/100 dilution of their sera. Data are shown as mean±SD.
* $P<0.01$.

not die of lupus lesions.

Although no data on toxicological findings are shown, a middle dose of 5 mg/kg led to no weight loss or toxic deaths. At a higher dose of 10 mg/kg, however, all mice showed some weight loss, and 1 of 10 mice (Expt I) and 2 of 9 mice (Expt II) died of apparent drug toxicity during the course of SGL administration. However, these doses did not elicit bone marrow suppression.

Discussion

The present paper details the efficacy of SGL on SLE-like lesions developing spontaneously in MRL/l mice. When the administration of SGL was started at 7 weeks of age, indices such as lymphadenopathy, leucocytosis, high anti-DNA antibody and BUN levels, and lupus nephritis were markedly suppressed at doses of 5 and 10 mg/kg. In the second group, SGL treatment was started at 13 weeks of age, a time when these mice already had circulating anti-DNA antibody and angiitis in their kidneys. This latter study was therefore a curative rather than a preventive one. Once again SGL at doses of 5 and 10 mg/kg significantly suppressed all the SLE-like indices. From these findings, SGL was found to be both prophylactically and curatively effective in MRL/l mice. Since the toxic findings indicated that a dose higher than 10 mg/kg was toxic under these administration schedules, the most effective dose is thought to be 5 mg/kg. The mechanisms responsible for the efficacy of SGL in immunodeficient animals must be examined.

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References

1) Umezawa, H.; S. Kondo, H. Iinuma, S. Kunimoto, Y. Ikeda, H. Iwasawa, D. Ikeda & T. Takeuchi: Structure of an antitumor antibiotic, spergualin. J. Antibiotics 34: 1622~1624, 1981
2) Umezawa, H.; M. Ishizuka, T. Takeuchi, F. Abe, K. Nemoto, K. Shibuya & T. Nakamura: Suppression of tissue graft rejection by spergualin. J. Antibiotics 38: 283~284, 1985
3) Nemoto, K.; J. Ito, M. Hayashi, F. Abe, Y. Ohtaka, T. Nakamura, T. Takeuchi & H. Umezawa: Effects of spergualin and 15-deoxyspergualin on graft-versus-host disease in mice. Transplant. Proc. 19: 3520~3521, 1987
4) Nemoto, K.; F. Abe, T. Takita, T. Nakamura, T. Takeuchi & H. Umezawa: Suppression of experimental allergic encephalomyelitis in guinea pigs by spergualin and 15-deoxyspergualin. J. Antibiotics 40: 1193~1194, 1987
5) Eaton, R. B.; G. Schneider & P. H. Schur: Enzyme immunoassay for antibodies to native DNA. Arthritis Rheum. 26: 52~62, 1983
6) Godfrey, D. G.; W. H. Stimson & J. Watson: Determination of anti-DNA autoantibody levels in mice by enzyme immunoassay. J. Clin. Lab. Immunol. 15: 223~225, 1984