SUPPRESSION OF HUMORAL IMMUNITY IN DOGS BY 15-DEOXYSPERGUALIN

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15-Deoxyspergualin (DSG)[1] is a derivative of spergualin which is a metabolite of *Bacillus laterosporus*[2], and has been found to have strong immunosuppressive activity in rodents[3,4]. In the present study, we investigated the effect of DSG on antibody formation to sheep red blood cells (SRBC) in dogs.

DSG was prepared by Takara Shuzo Co., Ltd.[5]. Female beagles were supplied by Nippon Kayaku Co., Ltd. The dogs (9.1~10.6 months old, 9.0~11.3 kg) were immunized with SRBC (Nippon Biosupp Center, Tokyo, Japan) as described in the legend of Table 1. Peripheral blood lymphocytes (PBL) were isolated by a simple density "cut" procedure in a Ficoll-Conray solution (Immuno-Biol. Lab., Tokyo, Japan). Plaque-forming cells (PFC) producing IgM were enumerated according to CUNNINGHAM and SZENBERG[6]. IgG-producing PFC were indirectly revealed with a 1/1,000 dilution of a rabbit anti-dog IgG antiserum (Miles Scientific, Naperville, U.S.A.). All data were analyzed by Student's t-test.

As shown in Table 1, IgM production in the primary response to SRBC was detected in the PBL isolated from control dogs ($n=6$). In five dogs, the production of IgM was maximal on day 7 after immunization. In one dog (No. 249), IgM production was maximal on day 4. When

Table 1. Inhibition of the primary response to SRBC in dogs by DSG.

DSG (mg/kg)	Dog No.	IgM-producing PFC (number/1×10^6 PBL)		
		Day 4	Day 7	Day 10
0	236	815	1,714	536
(Control group)	243	1,110	1,402	401
	244	618	1,394	339
	249	6,234	3,075	899
	250	1,322	2,629	486
	273	677	1,534	225
		1,796±894†	1,958±292	481±95
0.3	261	146	141	12
	263	223	509	22
	269	567	142	13
	270	1,390	86	13
	272	1,566	283	19
		778±296	232±76*	16±2*
1.2	203	95	36	18
	216	142	30	7
	229	31	207	13
		89±32	91±58*	13±3*

SRBC (1×10^{10}) were iv injected on day 0. DSG was iv administered once a day on day 0 to 9. PBL were isolated on the day shown in the table and assayed for IgM-producing PFC.
† Mean±SE. * $P<0.01$.

† Deceased.

Table 2. Inhibition of the secondary response to SRBC in dogs by DSG.

DSG (mg/kg)	IgG-producing PFC (number/1×10^6 PBL)
0	$1,375\pm439$†
1.2	40 ± 11*

The first injection of SRBC was carried out as described in the legend of Table 1. About 7 months later 2×10^8 SRBC was reinjected. DSG was iv administered once a day for 4 days from the day of the reinjection. PBL were isolated one day after the last administration and subjected to the assay of IgG-producing PFC. A group consisted of 3 dogs.

† Mean±SD. * $P<0.01$.

DSG was administered iv at doses of 0.3 and 1.2 mg/kg daily for 10 days from the day of the immunization, there was a significant inhibition of the primary response to SRBC that was observed on days 7 and 10. At the dose of 1.2 mg/kg the inhibition rate was more than 95% on days 7 and 10.

The effect of DSG on the secondary response to SRBC was further examined. The results are shown in Table 2. DSG at a dose of 1.2 mg/kg, markedly inhibited the production of IgG. Once again the inhibition rate was more than 95%.

Determination of anti-SRBC titer in sera from the dogs immunized with SRBC was carried out. Since lysis of SRBC of unknown origin was observed in the presence of about 1/100 dilution of serum from the immunized dogs, we have no data on serum antibody titer to SRBC. On the other hand, this phenomenon was not observed in normal dog serum.

Recently, we reported that DSG inhibited the primary response to SRBC in mice[4]. In the present study, DSG was found to inhibit not only the primary but also the secondary response to the antigen in dogs. DSG has been shown to prolong the survival of allografts in rats[3,4,7,8]. From our present findings we expect that DSG will be effective in aiding organ allo-transplantation in dog.

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