EFFECT OF DIKETOCORIOLIN B ON ANTIBODY FORMATION

Sir:

Most antitumor compounds have been known to be immunosuppressive, but in contrast, we found that intraperitoneal injection of diketocoriolin B (DKC)[1] increases the number of antibody-forming cells in mouse spleen.

Female *dd/Y* mice (5~6 weeks old, weighing 18~20 g) were immunized intravenously with sheep red blood cells (SRBC) and the numbers of antibody forming cells was determined 48 hours thereafter by the hemolytic plaque technique[2]. Results are shown in terms of average number of plaque forming cells (PFC) per 10^8 spleen cells. One mg of diketocoriolin B was dissolved in 0.2 ml of dimethylsulfoxide, then 1.8 ml of saline and a trace of Tween 80 (Wako Pure Chemical Ind. Ltd., Japan) were added. Each 0.2 ml of saline-diluted solution was injected to mice intraperitoneally.

As shown in Table 1, 125 mcg/mouse of DKC suppressed the primary immune responce. However, 31.2, 10 and 0.01 mcg/mouse of DKC markedly enhanced the response. In contrast with the effective dose in antitumor activity, it is apparent that DKC exhibits a stimulatory effect over a wide range of dosage. As reported previously by TAKEUCHI *et al.*[1], the LD_{50} of DKC by single intraperitoneal injection is 40 mg/kg, 0.75 mcg/ml inhibits growth of cultured YOSHIDA sarcoma cells *in vitro*, at daily intraperitoneal injection of 12.5~100 mcg/mouse/day for 10 days prolong the survival period of mice intraperitoneally inoculated with cells of L-1210 or carcinoma cells.

We tested the effect of DKC on the secondary immune response. Mice were immunized first with 10^4 or 10^5 SRBC, and 10^4 or 10^5 SRBC and DKC or DKC alone were injected 7 or 10 days later. Results are shown in Tables 2 and 3. As shown in Table 2, DKC also enhanced the response to the second injection of SRBC. Moreover, from 0.6 to 10 mcg of DKC alone increased

Table 1. Effect of diketocoriolin B on primary immune response

		PFC/10^8 cells	Ratio
I	SRBC* 10^8 i. v.	447.5 ± 23.0	1.00
	〃 〃 + DKC** 125 mcg i. p.	144.4 ± 21.8	0.32
	〃 〃 + 〃 31.2 mcg 〃	838.0 ± 51.3	1.87
	〃 〃 + 〃 7.8 mcg 〃	1,201.7 ± 44.8	2.69
	Control	4.3 ± 0.2	0.01
II	SRBC* 10^8 i. v.	587.6 ± 11.4	1.00
	〃 〃 + DKC** 10 mcg i. p.	930.7 ± 54.8	1.58
	〃 〃 + 〃 1 mcg 〃	873.5 ± 73.2	1.48
	〃 〃 + 〃 0.1 mcg 〃	1,148.3 ± 72.7	1.95
	〃 〃 + 〃 0.01 mcg 〃	966.5 ± 10.7	1.65
	〃 〃 + 〃 0.001 mcg 〃	607.5 ± 41.1	1.03
	Control	26.5 ± 2.5	0.05

* SRBC 10^8/0.1 ml/mouse ** Each dose of DKC/0.2 ml/mouse

Table 2. Effect of diketocoriolin B on secondary immune response. I

Primary	Secondary*	PFC/10^8 cells**	Ratio
SRBC 10^5 i. v.	——	47.9 ± 2.6	— 1.00
〃 〃	SRBC 10^5 i. v.	6,933.5 ± 311.2	1.00 —
〃 〃	〃 〃 + DKC 10 mcg i. p.	12,420.0 ± 242.5	1.79 —
〃 〃	—— + DKC 10 mcg 〃	197.6 ± 33.6	— 4.05
〃 〃	—— + 〃 2.5 mcg 〃	719.3 ± 8.8	— 14.73
〃 〃	—— + 〃 0.6 mcg 〃	197.0 ± 1.4	— 4.03
——	—— + 〃 10 mcg 〃	18.2 ± 3.7	— —
Control	Control	20.8 ± 5.7	— —

SRBC/0.1 ml/mouse Each dose of DKC/0.2 ml/mouse
* 7 days after the primary injection ** 48 hours after the secondary injection

Table 3. Effect of diketocoriolin B on secondary immune response. II

Primary	Secondary*	PFC/10^8 cells**	Ratio	
SRBC 10^4 i. v.	——	38.9 ± 7.8	1.00	—
〃	SRBC 10^4 i. v.	49.7 ± 8.0	—	1.00
〃	SRBC 10^4 i. v. + DKC 10 mcg i. p.	187.0 ± 6.8	—	3.75
〃	〃 〃 + 〃 1 mcg 〃	490.5 ± 30.0	—	9.83
〃	〃 〃 + 〃 0.1 mcg 〃	799.8 ± 55.1	—	16.03
〃	〃 〃 + 〃 0.01 mcg 〃	283.5 ± 16.7	—	5.68
〃	—— + DKC 10 mcg i. p.	293.4 ± 20.3	7.54	—
〃	—— + 〃 1 mcg 〃	147.3 ± 17.4	3.79	—
〃	—— + 〃 0.1 mcg 〃	107.3 ± 10.6	2.77	—
〃	—— + 〃 0.01 mcg 〃	54.2 ± 4.3	1.39	—
Control	——	30.6 ± 2.7	—	—

SRBC 10^4/0.1 ml/mouse Each dose of DKC/0.2 ml/mouse
* 10 days after the primary injection ** 48 hours after the secondary injection

the numbers of plaque forming cells. Comparing the numbers of PFC in mice injected with DKC alone to that of primed mice without the second injection, DKC increased PFC 4~14 times. These observations were confirmed in another experiment, testing the effect on mice immunized with a smaller amount of antigen (Table 3). Mice were first given 10^4 SRBC and then 10 days later they were injected with 10^4 SRBC and DKC or DKC alone. The poor response in primed mice which received a small quantity of antigen was markedly elevated by DKC in dosages as low as 0.1~1 mcg per mouse.

KUNIMOTO *et al.*[3] observed that the mode of action of DKC on mammalian cells involves inhibition of Na-K-ATPase causing efflux of intracellular amino acids and potassium ion and inhibiting their uptake. It is also known that cell membrane effects of chlorpromazine[4], phytohemagglutinin[5], antilymphocyte serum[6] and Ca^{++} ion[7] alter antibody forming abilities. The mechanism of such action is however not clear. The mechanism of action of DKC to increase antibody forming cells is now under study.

MASAAKI ISHIZUKA
HIRONOBU IINUMA
TOMIO TAKEUCHI
HAMAO UMEZAWA

Institute of Microbial Chemistry,
Shinagawa-ku, Tokyo, Japan

(Received February 14, 1972)

References

1) TAKEUCHI, T.; S. TAKAHASHI, H. IIMUNA & H. UMEZAWA: Diketocoriolin B, an active derivative of coriolin B produced by *Coriolus consors*. J. Antibiotics 24: 631~635, 1971
2) JERNE, N. K.; A. A. NORDIN & C. HENRY: The agar plaque technique for recognizing antibody-producing cells. Cell-bound Antibodies. B. AMOS and H. KOPROWSKI ed. pp. 109~122, Wister Institute Press, Philadelphia, 1963
3) KUNIMOTO, T.; M. HORI & H. UMEZAWA: Mechanism of action of diketocoriolin B. (to be published)
4) BRAUN, W. & M. NAKANO: The role of cell breakdown products in the stimulation of immune responses. Symp. Series Immunobiol. Standard. 6: 227~234, Karger, New York, 1967
5) SPREAFICO, F. & E. M. LERNER, II: Suppression of the primary and secondary immune response of the mouse by phytohemagglutinin. J. Immunol. 98: 407~416, 1967
6) YAJIMA, Y. & W. BRAUN: Non-specific triggering of antibody production by poly-A and poly-U in the presence of lymphocyte serum. Fed. Proc. 28: 431, 1969
7) BRAUN, W.; M. ISHIZUKA & P. SEEMAN: Suppression and enhancement of antibody formation by alteration of Ca^{2+} levels. Nature 226: 945~946, 1970