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Short Communication

TUMOUR IMMUNOPROPHYLAXIS IN MICE USING GLUTARALDEHYDE-TREATED SYNGENEIC MYELOMA CELLS

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ATTEMPTS to induce antitumour protection with syngeneic tumour cells treated with glutaraldehyde (GA) have been reported by numerous workers. Induction of antitumour protection was reported in some cases (Csaba, 1967; Powell, 1975; Sanderson & Frost, 1974; Frost & Sanderson, 1975; Frost *et al.*, 1976; Kataoka *et al.*, 1975, 1977*a*, *b*, 1978, 1979*a*, *b*, *c*, *d*; Tomecki, 1979) whereas in other systems no protection was achieved (Kluchareva *et al.*, 1978; Price *et al.*, 1979). The use of GA was based on its property as a stabilizer of the cell surface by virtue of its activity as a protein cross-linking reagent (Richard & Knowles, 1968). It has

TABLE.—*Immunization of BALB/c mice with GA-treated MOPC-315 plasmacytoma cells*

			Immunization procedure**		Take			Mortality††	
Pool Number	No. of Expts	No. of groups*	No. inj.	First injection (%GA)	+ve/ total	MTD† (days)	*P*†††	MTM (days)	*P*
I	5	9	2 or 4	0·08§	89/116	27·7	< 0·001	40·4	< 0·001
		5			52/52	14·4		30·1	
II	3	9	1	0·08	51/66	23·0	< 0·01	37·8	< 0·01
		3			32/32	14·0		29·9	
III	3	7	2 or 4	0·03 to	48/58	24·1	< 0·001	37·9	< 0·001
		3		0·06§§	29/30	15·3		29·5	
IV	2	4	1	0·06	18/25	23·8	< 0·05	38·7	< 0·05
		2			20/20	15·7		31·8	
V	3	5	1	0·04	27/33	23·0	< 0·02	38·2	< 0·02
		3			29/30	15·8		30·5	
VI	5	7	1	0·02	56/57	15·2	NS	31·0	NS
		5			49/50	15·5		30·4	
I	5	9	2 or 4	0·08	89/116	27·7	< 0·05	40·4	NS
II	3	9	1	0·08	51/66	23·0		27·8	

* 5–20 mice per group.

** All injections were given s.c.; cells treated with 0·02% GA were used for last immunization; immunization: 5×10^5 to 5×10^6 cells per mouse; challenge: 10^4 viable tumour cells.

† MTD—mean time to tumour detection.

†† All tumour-bearing animals died during observation; MTM—mean time of mortality.

§ Cells treated with 0·06% GA and 0·04% GA were used subsequently in 6 groups receiving intermediate injections.

§§ Cells treated with 0·03% GA were used for the intermediate injection in groups receiving 3 injections (2 groups); intermediate injections of 0·04% GA-treated and 0·03% GA-treated cells were given in groups receiving 4 injections (2 groups).

††† *P*—degree of significance was calculated by Student's *t* test; significant protection was obtained in 8/9 individual groups of Pool I, 5/9 of Pool II, 4/7 Pool III, 2/4 of Pool IV, 1/5 of Pool V and 0/7 of Pool VI.

also been claimed that modification of cells by GA reduced the antibody response, whereas the cellular response was maintained and even increased (Dennert & Tucker, 1972; Parish, 1972). Preferential elicitation of cell-mediated immunity was assumed to be advantageous for induction of specific protective immunization against tumours (Mitchison, 1970).

The present study was undertaken to investigate the possibility of induction of antitumour protection against murine myeloma MOPC-315 in syngeneic BALB/c mice by use of GA-treated cells. The MOPC-315 myeloma cell line is derived from primary plasmacytoma tumour induced in mice of the BALB/c strain by i.p. administration of Bayol F, develops exclusively in this strain and is characterized by its ability to secrete anti-TNP IgA λ_2 immunoglobulin (Eisen *et al.*, 1968). This tumour cell line is highly tumorigenic by either i.p. or s.c. injection, and has been reported as weakly immunogenic (Williams & Kruger, 1972).

Suspensions of MOPC-315 cells prepared from s.c. induced tumours were used in all the experiments. The treatment with GA was performed by mixing volumes of 0·2 ml of cell suspensions (2×10^7 viable cells/ml) in PBS at pH 7·2 with 1·8 ml GA solution (TAAB, England), for 10 min at room temperature. The treated cells were washed by 3 subsequent centrifugations at 100 *g* for 7 min at 4°C. Packed cells were resuspended either in Eagle's medium for thymidine (dT) incorporation measurements (Diamantstein & Ulmer, 1975) or in PBS for injections. The results in Fig. 1A show that treatment of cells by a solution of 0·00125% GA led to a marked decrease in (dT) incorporation whilst GA concentrations of 0·01–0·08% almost completely inhibited the incorporation. As shown in the same figure, the viability of cells, as measured by trypan-blue dye exclusion, was less affected by GA; marked reduction was obtained only at a concentration of at least 0·06% GA. MOPC-315 cells treated with less than 0·01% GA retained their ability to induce tumours. Partial loss of this ability (delay in development of tumours and reduction in mortality) was seen after treatment with 0·01–0·02% GA (Fig. 1B). Complete loss of the capacity to induce tumour was achieved by treatment with 0·06–0·08% GA (results not shown here). In this particular case, the cell suspension used was the same as in the experiment illustrated in Fig. 1A.

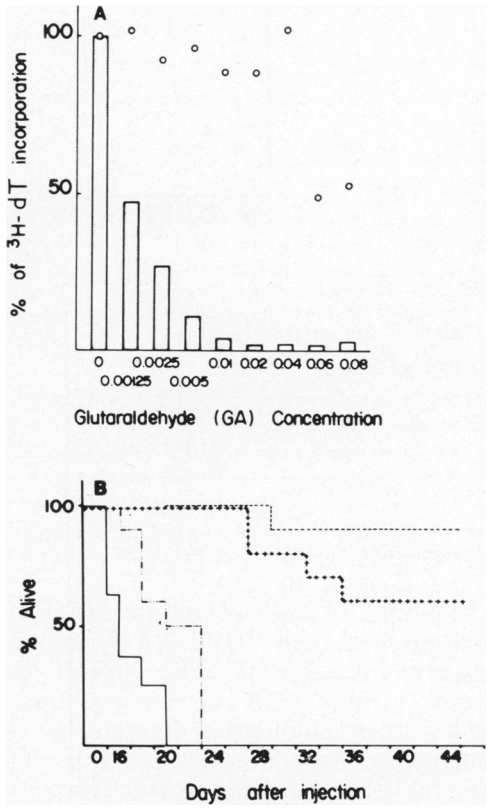

FIG. 1.—Effect of treatment with various concentrations of GA on MOPC-315 cells originated from s.c. induced tumours; 4×10^6 cells/0·2 ml were mixed with 1·8 ml of GA solution and incubated for 10 min at room temperature. A—GA-treated cells subcultured for 24 h; [^{3}H]-dT added 6 h before the end of incubation time. □—% of [^{3}H]-dT incorporation (levels in untreated cultures (2797 ± 496) taken as 100%). ○—% viability (levels in untreated cultures taken as 100%). B—Tumorigenicity of GA-treated cells; 5×10^5 cells per mouse s.c.; 8–10 mice per group. GA concentration (%): – – – 0·02; + + + 0·01; ·–·–·—·– 0·005; —— none.

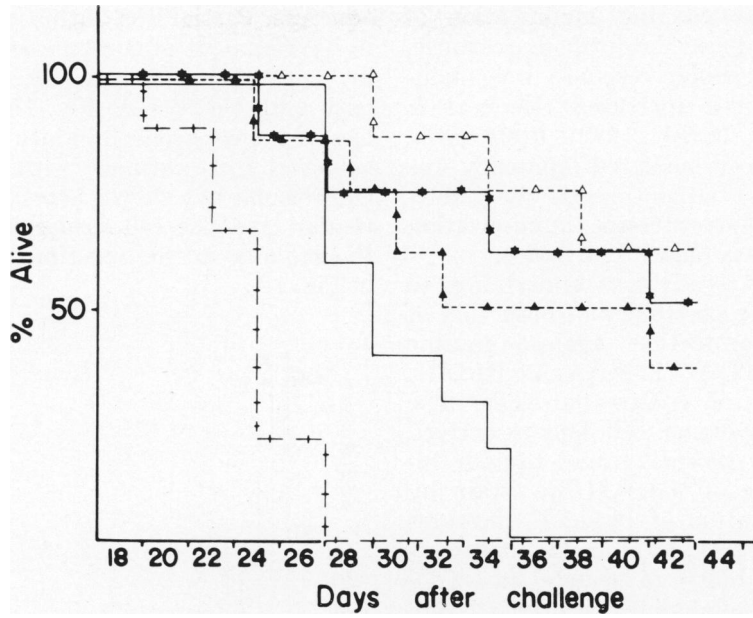

FIG. 2.—Protective effect of s.c. injection (21 and 7 days before challenge) with GA-treated MOPC-315 cells (5×10^5/injection) originated from s.c. tumour; Challenge of 10^4 viable cells given s.c. 7 days after the last immunizing injection. GA concentrations varied (as indicated) between 0·02 and 0·08%.

*Immunization schedule**

—*———*———*— 0·08(28)–0·02(7)–challenge
– –▲– – –▲– – –▲– – 0·06(28)–0·02(7)–challenge
– –△– – –△– – –△– – 0·04(28)–0·02(7)–challenge
—+— —+— —+— 0·02(7)–challenge
——————————— untreated–challenge

* The death rate in the group immunized by one injection of 0·02% GA-treated cells was significantly higher ($P < 0·001$) than in the control unimmunized group.

Induction of antitumour protection by immunization with MOPC-315 GA-treated cells was investigated under various conditions, including GA concentrations used for treatment, number of immunizing injections, number of cells per injection, interval between injections and number of days between the last immunizing injection and challenge. The results summarized in the Table demonstrate that immunization with GA-treated MOPC-315 cells induced partial, though significant, protection against challenge with 10^4 tumour cells. The antitumour protection was more marked in groups receiving more than one immunizing injection, especially if the first injection was with cells treated with 0·08% GA. A typical experiment is illustrated in Fig. 2.

The results show that the effect of GA was directly proportional to its concentration: firstly, impairment of dT incorporation, secondly, loss of tumorigenic ability and finally, change in trypan-blue dye exclusion. Correlation between the concentration of GA and the effect on tumour cells has also been reported by other workers (Bubbers & Henney, 1975; Kataoka *et al.*, 1975; Price *et al.*, 1979). Immunization with MOPC-315 cells treated with GA afforded partial protection against tumorigenic challenge. It would be of interest to devise more optimal schedules for immunization, and to clarify the nature of the immune response in protected and unprotected animals submitted to a similar schedule of immunization.

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