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INCREASED CYTOLYTIC EFFECT OF IMMUNE LYMPHOCYTES IN A SYNGENEIC TUMOUR SYSTEM FOLLOWING SIMPLE PURIFICATION PROCEDURES

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SYNGENEIC or allogeneic tumour cells induce immune responses which lead to the formation of cytotoxic thymus dependent lymphocytes that can be detected in lymphoid organs, blood and peritoneal exudates. However, in weak syngeneic systems it is often difficult to detect *in vitro* cytotoxicity, the possible reasons being either the low relative frequency of effector cells or their blocking by antigens. Since the cytotoxic lymphocytes are mainly of low density in the early phase of the immune response, their functional separation from irrelevant cell populations, such as phagocytic cells or high density small lymphocytes, can be achieved by simple physical methods (Pelet *et al.*, 1971; Shortman, Cerottini and Brunner, 1972). Moreover, cytotoxicity of immune lymphocytes has been shown to increase following overnight incubation (Landazuri and Herberman, 1972) or repeated washing (Currie and Basham, 1972).

The formation of cytotoxic lymphocytes was studied in a weak syngeneic tumour system. C57BL mice were given two i.p. injections of 0.5×10^6 and 50×10^6 irradiated (3000 rad) EL4 (C57BL) cells, respectively, with a 3-week interval (modified from Martin *et al.*, 1971). The peak of the secondary immune response was found to be on the 7th day. Cytotoxicity of recipient lymphocytes was measured by the chromium release assay technique (Brunner *et al.*, 1968).

When immune spleen cells were directly tested against EL4 cells, the mean value of the specific release of chromium at 20 hours with a lymphocyte target cell ratio of 100 : 1 was 10% (range 8–15). When the immune spleen cells having a density less than 1·08 g/cm^3 were selected by BSA gradient separation (modified from Shortman, Williams and Adams, 1972), the cytotoxicity was increased to a mean of 21% (range 19–25). When the immune spleen cells were incubated at 37°C for 24 hours and then tested against EL4 cells, the cytotoxicity was 25% (range 21–37). However, when the immune spleen cells were first incubated for 24 hours and then purified by BSA gradient, the cytotoxicity was increased to 52% (range 40–60). Values of a representative experiment are given in Table I. The specificity of the reaction was ascertained by testing against

TABLE I

Pretreatment of immune spleen cells	Target cells	Specific release of chromium in 20 hours at lymphocyte/target cell ratio of 100 : 1	30 : 1	10 : 1
Nil	EL4	15	10	6
BSA purification	EL4	25	20	13
24 hour incubation	EL4	29	19	11
24 hour incubation; then BSA purification	EL4	60	38	20
BSA purification; then antitheta serum and complement	EL4	8	6	4
24 hour incubation; then BSA purification	P-815	7	4	2

TABLE II

Immune cells	Pretreatment	Target cells	Specific release of chromium in 20 hours at effector/target cell ratio of 100 : 1	30 : 1	10 : 1
Spleen cells	Nil	EL4	15	10	6
Peritoneal cells	Nil	EL4	30	24	17
Peritoneal cells	Carbonyl iron	EL4	45	36	28
Peritoneal cells	24 hour incubation	EL4	50	39	28
Peritoneal cells	Carbonyl iron	P-815	8	4	1
Peritoneal cells	24 hour incubation	P-815	10	6	3

unrelated target cells such as P-815, where cytolysis was less than 10%. Cytotoxicity appeared to be predominantly a T cell dependent function because pretreatment of spleen cells with antitheta serum and complement, almost completely abolished the activity.

Immune peritoneal cells were more cytotoxic than the spleen cells. Moreover, their cytotoxicity was increased over three-fold when the phagocytic cells were removed from the peritoneal cell population by treatment with carbonyl iron or when the adherent cells were separated by means of their adherence to glass. A representative experiment with peritoneal cells is shown in Table II.

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