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Human myeloma cells and their strong stimulating capacity in 'one-way' mixed lymphocyte reaction: a comparative study with leukaemic B lymphoid cells

T. HAN, B. DADEY, J. MINOWADA & D. W. GOLDE *Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York and University of California, Los Angeles School of Medicine, Los Angeles, California, U.S.A.*

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Summary. Cultured human myeloma cells (ARH-77, RPMI-8226 and U-266), like leukaemic B lymphoid cells, consistently exerted a strong stimulating capacity on allogeneic lymphocytes in the 'one-way' mixed lymphocyte reaction. An optimal stimulation was seen when a 1:1 ratio or 1:2 ratio of responding cell: stimulating cells of each cell line was utilized. The stimulating capacity of ARH-77 or RPMI-8226 cells was significantly diminished when a 1:4 ratio of responding cells: stimulating cells was utilized. Fresh bone marrow cells containing more than 80% plasma cells from a patient with multiple myeloma, on the other hand, failed to exert the stimulating capacity on two occasions. The striking difference between cultured myeloma cells and fresh plasma cells is that the Ia-like antigen is present on cultured myeloma cells, and this antigen is absent on fresh plasma cells. The relationship between the Ia-like antigen and the stimulating capacity in 'one-way' mixed lymphocyte reaction is discussed.

INTRODUCTION

It has long been demonstrated that cultured human normal B lymphoid cells possess a strong stimulating capacity on allogeneic as well as autologous human lymphocytes in the 'one-way' mixed lymphocyte reaction (Han, 1972; Han, Sokal & Moore, 1972; Hardy, Ling & Knight, 1969). Recent studies reported by us and others demonstrated that cultured human leukaemic B lymphoid cells exert a significant stimulation while leukaemic T lymphoid cells exert no stimulation on allogeneic normal lymphocytes (Han & Minowada, 1973; Royston, Graze & Pitts, 1974; Pauly, Minowada, Han & Moore, 1975; Han, Dadey & Minowada, 1977).

We have established the first human myeloma cell line (RPMI-8226) in our Institute, and the two other myeloma cell lines (ARH-77 and U-266) were established by others. The present study describes the stimulating capacity in the 'one-way' mixed lymphocyte reaction of these cultured human myeloma cell lines and the comparison of their stimulating capacity with that of cultured human leukaemic B lymphoid cell lines established from patients with various types of B-cell malignancy.

Correspondence: Dr Tin Han, Roswell Park Memorial Institute, New York State Department of Health, 666 Elm Street, Buffalo, New York 14263, U.S.A.

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MATERIALS AND METHODS

Myeloma cell lines

Three myeloma cell lines (ARH-77; RPMI-8226; and U-266) were used for the source of stimulating cells in the 'one-way' mixed lymphocyte reaction. All cell lines were originally established from the bone marrow cells

and/or peripheral blood cells of patients with multiple myeloma (Matsuoka, Moore, Yagi & Pressman, 1967; Nillson, Bennich, Johansson & Ponten, 1970; Burk, Drewinko, Trujillo & Ahearn, 1978). The RPMI-8226 cells grew on the glass surface and produced only λ light chain immunoglobulins. The U-266 cells grew in suspension and produced λ light chain and ε heavy chain immunoglobulins. The ARH-77 cells also grew in suspension and produced κ light chain and γ heavy chain immunoglobulins. The growth patterns of these three cell lines were quite similar with doubling times ranging from 24 to 36 h. For comparison of stimulating capacity of myeloma cells with leukaemic B lymphoid cells, the following leukaemic B lymphoid cell lines were used: the BALL-1 and BALM-2 cell lines were originally established from patients with acute lymphoblastic leukaemia (ALL); the BALM-3, BALM-4 and BALM-5 cell lines were established from a patient with malignant lymphoma (ML); the B35M was established from a patient with Burkitt's lymphoma (BL); the PR and RI cell lines were established from patients with hairy cell leukaemia (HCL) (Minowada, Tsubota, Nakazawa, Greaves, Janossy, Koshiba, Kubonishi, Han, Higby, Freeman & Sinks, 1978; Lok, Han & Minowada, submitted for publication; Saxon, Stevens, Quan & Golde, 1978).

Cell surface and antigen marker analyses of these cultured myeloma cells were performed at the time of this study. Rosetting procedures were used for detection of the E and EAC receptors (Minowada, Ohnuma & Moore, 1972; Minowada, Tsubota, Nakazawa, Srivastava, Huang, Oshimura, Sonta, Han, Sinks & Sandberg, 1977). Surface membrane immunoglobulin (SmIg) was detected by the direct membrane immunofluorescence test using fluorescein-conjugated goat antibody specific for each immunoglobulin chain. The indirect membrane immunofluorescence test using a panel of specific rabbit antisera and fluorescein-conjugated goat anti-rabbit globulin reagent was carried out to identify the human thymus-leukaemia antigen (HTLA), the Ia-like B-cell associated antigen (Ia-like) and the antigen specific for the common form of acute lymphoblastic leukaemia (cALL). A detailed method for each antigen identification has previously been published (Greaves, Brown, Ropson & Lister, 1975; Schlossman, Chess, Humphreys & Strominger, 1976; Tsubota, Minowada, Nakazawa, Sinks, Han, Higby & Pressman, 1977).

'One-way' mixed lymphocyte reaction

This test was performed, using a slightly modified whole-blood method developed in this laboratory (Pauly, Sokal & Han, 1973). In brief, heparinized peripheral blood from healthy subjects was mixed with RPMI 1640 culture medium containing 100 units of penicillin and 50 μg of streptomycin per millilitre, at a ratio of 1:20. Three millilitre aliquots of cell suspension, containing approximately 3×10^5 lymphocytes, were transferred to 16×125 mm disposable plastic culture tubes. Stimulating cells were inactivated by Mitomycin-C treatment (25 μg per ml of cell suspension at 37° for 30 min). Stimulating cell suspensions (0·5 ml) containing various cell concentrations were added to responding cell cultures, making a 1:1, 1:2, or 1:4 ratio of responding lymphocytes to stimulating cells. Each experiment also included single cell cultures (responding cell cultures with or without PHA and stimulating cell cultures) in addition to mixed-cell culture. The experiments were carried out in duplicate. Culture tubes with loose-fitting caps were incubated at 37° in a humidified atmosphere of 5% CO_2 in air for 7 days. One microcurie of [^{3}H]-thymidine (specific activity, 2·0 Ci/mmol) was added to each tube 24 h prior to harvesting the cells. Incorporation of [^{3}H]-thymidine into DNA was measured as previously described (Pauly *et al.*, 1973). 'One-way' mixed lymphocyte reaction was expressed as the stimulation index (SI) which is counts per minute (c.p.m.) in mixed-cell culture minus c.p.m. in stimulating cell culture, divided by c.p.m. in responding cell culture. An SI of more than 2 was considered to be significant.

RESULTS

Surface and antigen markers of human myeloma cell lines and human leukaemic B lymphoid cell lines are presented in Table 1. All myeloma cell lines had similar surface and antigen marker expressions except for the EAC receptors. The EAC receptors were positive in ARH-77 cells while these receptors were negative in RPMI-8226 and U-266 cells. All five leukaemic B lymphoid cell lines (BALL-1, BALM-2, B35M, PR and RI), established from patients with B-cell ALL, B-cell BL or B-cell HCL had identical surface and antigen marker expressions. The EAC receptors were positive in each of these five cell lines. The remaining three leukaemic B lymphoid cell lines (BALM-3, BALM-4 and BALM-5), established from a patient with B-cell ML, had negative EAC receptors and positive cALL antigens, unlike other B lymphoid cell lines.

The results of stimulating capacity of three cultured

Table 1. Surface and antigen markers of human multiple myeloma cell lines and human leukaemic B lymphoid cell lines

Cell lines	Donor origin*	Surface and antigen markers					
		E	EAC	SmIg	HTLA	Ia-like	cALL
Multiple myeloma cell lines							
ARH-77	MM	−	+	+(κ,γ)	−	+	−
RPMI-8226	MM	−	−	+(λ)	−	+	−
U-266	MM	−	−	+(λ,ε)	−	+	−
Leukaemic B lymphoid cell lines							
BALL-1	B-cell ALL	−	+	+	−	+	−
BALM-2	B-cell ALL	−	+	+	−	+	−
BALM-3	B-cell ML	−	−	+	−	+	+
BALM-4	B-cell ML	−	−	+	−	+	+
BALM-5	B-cell ML	−	−	+	−	+	+
B35M	B-cell BL	−	+	+	−	+	−
PR	B-cell HCL	−	+	+	−	+	−
RI	B-cell HCL	−	+	+	−	+	−

* MM, multiple myeloma; ALL, acute lymphoblastic leukaemia; ML, malignant lymphoma; BL, Burkitt's lymphoma; HCL, hairy cell leukaemia.

myeloma cell lines are represented in Table 2. All myeloma cell lines consistently exerted a strong stimulation on allogeneic normal lymphocytes. An optimal stimulation was seen when a 1:1 ratio or 1:2 ratio of responding cells:stimulating cells of each cell line was utilized. The stimulating capacity of ARH-77 cells or RPMI-8226 cells was significantly diminished when a 1:4 ratio of responding cells:stimulating cells was utilized ($P < 0.01$ or $P < 0.05$, respectively).

Table 3 shows the stimulating capacity of cultured leukaemic B lymphoid cell lines established from patients with various types of B-cell neoplastic diseases. Each and every B-cell line exerted a strong stimulating capacity on allogeneic normal lympho-

Table 2. Stimulating capacity of cultured human multiple myeloma cells in 'one-way' mixed lymphocyte reaction

Stimulating	Ratio of responding cells: stimulating cells	No. of expt	'One-way' mixed lymphocyte reaction, SI (Mean ± SE)
Multiple myeloma cell lines			
ARH-77	1:1	22	71·9 ± 13·6*
	1:2	21	64·1 ± 9·7
	1:4	13	18·8 ± 5·4*
U-266	1:1	23	39·3 ± 7·9
	1:2	18	71·4 ± 22·0
	1:4	8	40·8 ± 10·5
RPMI-8226	1:1	23	28·0 ± 5·5†
	1:2	17	21·5 ± 6·7
	1:4	8	6·0 ± 2·4†

* $P < 0.01$, † $P < 0.05$ by the Student's t test.

Table 3. Stimulating capacity of cultured human leukaemic B lymphoid cells in 'one-way' mixed lymphocyte reaction

Stimulating cell lines	Ratio of responding cells: stimulating cells	No. of expt	'One-way' mixed lymphocyte reaction, SI (Mean ± SE)
BL (B35M)	1:1	41	88·0 ± 10·5
ALL (BALL-1 and BALM-2)	1:1	15	13·9 ± 4·9
ML (BALM-3, BALM-4 and BALM-5)	1:1	6	22·9 ± 4·7
	1:2	6	29·2 ± 6·7
	1:4	6	27·3 ± 6·0
HCL (PR and RI)	1:1	6	163·7 ± 32·2
	1:2	6	163·4 ± 33·6
	1:4	5	116·8 ± 30·3

cytes. The HCL cells or BL cells possessed a very strong stimulation while the ALL cells or ML cells possessed a lesser but significant stimulation.

In two experiments, we investigated the stimulating capacity of fresh myeloma cells (bone marrow), obtained from a patient with multiple myeloma on allogeneic normal lymphocytes in the 'one-way' mixed lymphocyte reaction. The bone marrow cells contained more than 80% plasma cells. Surface and antigen markers of these fresh multiple myeloma cells were similar to established myeloma cells except that the Ia-like antigens were not detectable in fresh plasma cells. Fresh plasma cells were found to be non-stimulatory to allogeneic lymphocytes in both instances (not shown in Table).

DISCUSSION

It is now well known that fresh or cultured leukaemic T lymphoid cells possess no stimulating capacity, while leukaemic B lymphoid cells, on the other hand, possess a strong stimulating capacity in the 'one-way' mixed lymphocyte reaction (Han & Minowada, 1973; Royston *et al.*, 1974; Leventhal, Leung, Johnson & Poplack, 1977; Han *et al.*, 1977). To our knowledge, there is no published report on stimulating capacity of fresh or cultured myeloma cells on allogeneic lymphocytes. The present study unequivocally demonstrated that cultured myeloma cells, like leukaemic B lymphoid cells, exerted a strong stimulating capacity on allogeneic normal T lymphocytes. In contrast, fresh myeloma cells exerted no stimulation in two experiments. It is of interest that the surface and antigen markers of fresh myeloma cells are identical to cultured myeloma cells except for the absence of Ia-like antigens. It has recently been demonstrated that the Ia-like antigens are shown to be absent in most terminally differentiated immunoglobulin-producing plasma cells from patients with multiple myeloma and that the majority of plasma cells induced by pokeweed mitogen continue to express the Ia-like antigens (Halper, Fu, Wang, Winchester & Kunkel, 1978). These observations support the hypothesis that the Ia-like antigens are differentiation antigens for B cells and are usually lost by the terminal stage of B-cell development.

It has been reported that at least some of I γ-associated murine lymphoid cell surface antigens (Ia antigens) are preferentially expressed on B lymphoid cells (Sachs & Cone, 1974) and that these Ia antigens may play a major role as stimulators in murine mixed lymphocyte reaction (Fathman, Handwerger & Sachs, 1974; Lozner, Sachs, Shearer & Terry, 1974). Human counterpart Ia-like antigens are shown to be present in normal or leukaemic B lymphoid cells, leukaemic non-T/non-B lymphoid cells and absent in normal or leukaemic T lymphoid cells and leukaemic myeloid cells (Schlossman *et al.*, 1976; Han *et al.*, 1977; Han & Minowada, 1978). Observations in the present study that cultured myeloma cells with Ia-like antigens possess a stimulation while fresh myeloma cells without Ia-like antigens possess no stimulation, suggest that there is correlation between the Ia-like antigens and the stimulation in the 'one-way' mixed lymphocyte reaction. It has also been reported that antiserum to Ia-like antigens can completely block the 'one-way' mixed lymphocyte reaction, suggesting that the Ia-like

antigens are responsible for the stimulation in mixed lymphocyte reaction in man (Cresswell & Geir, 1977). We, on the other hand, observed little or no blocking effect of anti-Ia-like antiserum on the stimulating capacity of Ia-like antigen-positive cultured leukaemic non-T/non-B lymphoid cells (Han & Minowada, 1978). We also recently reported that cultured leukaemic myeloid cells (K562) which are negative for Ia-like antigens, are excellent stimulators while cultured non-T/non-B lymphoid cells (REH and KM-3), which are positive for Ia-like antigens are incapable of stimulation in the 'one-way' mixed lymphocyte reaction (Han *et al.*, 1977; Han & Minowada, 1978; Han, Dadey & Minowada, 1978). These observations suggest that the Ia-like antigens may not be solely responsible for the stimulation or that Ia-like antigens and the stimulation in the mixed lymphocyte reaction may be under control of closely linked but separate genetic systems in man.

Lack of stimulating capacity of fresh myeloma cells on allogeneic normal lymphocytes, observed in the present study, is of interest. It should be emphasized that we carried out only two experiments utilizing fresh myeloma cells from a patient with multiple myeloma, who had unusually high percentage of myeloma cells in bone marrow. Because of lack of technique for isolation of myeloma cells from bone marrow B cells at the present time and of a rarity of multiple myeloma patients with very high myeloma cells, we were unable to carry out further experiments for clarification of stimulating capacity of fresh myeloma cells. It has recently been reported that two non-T/non-B lymphoid cell lines (REH and KM-3) possess no stimulating capacity in the 'one-way' mixed lymphocyte reaction and these REH and KM-3 cells act as suppressor cells on normal T lymphocyte response to mitogen as well as allogeneic cells (Han, Dadey & Minowada, 1979). Since we did not carry out experiments, utilizing mixed (fresh and cultured) myeloma cells in the present study, the possibility that the failure of fresh myeloma cells to stimulate is attributable to a suppressor cell activity cannot be ruled out entirely.

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