In Vitro Lymphocyte Response to Autologous Cultured Lymphoid Cells (35409)

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Cultured lymphoid cell lines have been established from the peripheral blood of normal individuals and of patients with various types of neoplastic disease (1). Some cell lines have been successfully maintained for several years. The chromosomal karyotypes of established cell lines are normal initially; some cell lines have developed stable marker chromosomes or varying chromosomal abnormalities, but many remain entirely normal (2). Leukocyte antigens (HL-A system) of the cultured lymphoid cells are usually identical with those of autologous circulating lymphocytes at the beginning of establishment of cell lines. However, extra leukocyte antigens have been found in some culture cell lines after several months.

Bain *et al.* (3) reported in 1964 that when peripheral lymphocytes of two unrelated individuals are cultured together, some of the cells undergo blastic transformation. Ivanyi *et al.* (4) found that there is a good correlation between the intensity of the mixed cell reaction and differences among the strong transplantation antigens. The blastogenic effect of homologous circulating lymphocytes is rather weak as compared to that of phytohemagglutinin (PHA). In our experience, cultured homologous lymphoid cells exert significantly stronger effects, giving responses approaching those to PHA. Similar findings were reported by Hardy *et al.* (5). We have found only one report describing lymphocyte stimulation by cultured autologous lymphoid cells. Steel and Hardy (6) established cell lines from two patients with infectious mononucleosis. After recovery from the disease, circulating lymphocytes from both individuals were stimulated by the autologous cell lines which had been established during active disease.

The present report describes the response of circulating lymphocytes of 10 subjects to autologous cultured cells.

Materials and Methods. Cultured cells were obtained from established cell lines, grown in RPMI no. 1640 culture medium with 10–20% fetal calf serum. These cell lines had been in continuous culture for 1–42 months prior to these experiments. Three of five cell lines from normal individuals had been established for 34–42 months. A marker chromosome was seen in two of these three cell lines, and the third showed a change in karyotype from diploid to tetraploid; two lines contained EB viral antigen, detected in less than 1% of cultured cells. The other two cell lines from normal individuals had been in culture for 1 and 18 months, respectively; both had a normal diploid karyotype and neither contained viral antigen. Five cell lines from patients with neoplastic disease (2 malignant melanoma; 1 renal carcinoma; 1 breast carcinoma; and 1 reticulum cell sarcoma) had been in culture for 1–10 months. All of these five cell lines had a normal diploid karyotype, and only one line contained EB viral antigen, detected in less than 1% of cultured cells.

Circulating lymphocytes were obtained according to a method previously described (7), with the exception that RPMI no. 1640 culture medium without fetal calf serum or antibiotics was used. Cultured cells were washed three times with plain medium before use. Individual culture tubes contained 10^6 lymphocytes (reactive cells) and 2×10^5 or 10^5 cultured cells (target cells), or 10^6 or 2×10^5 homologous lymphocytes (target cells), in 2 ml of culture medium and 0.2 ml of autologous plasma. The target cells were irradiated with 5000–6000 R to create a one-

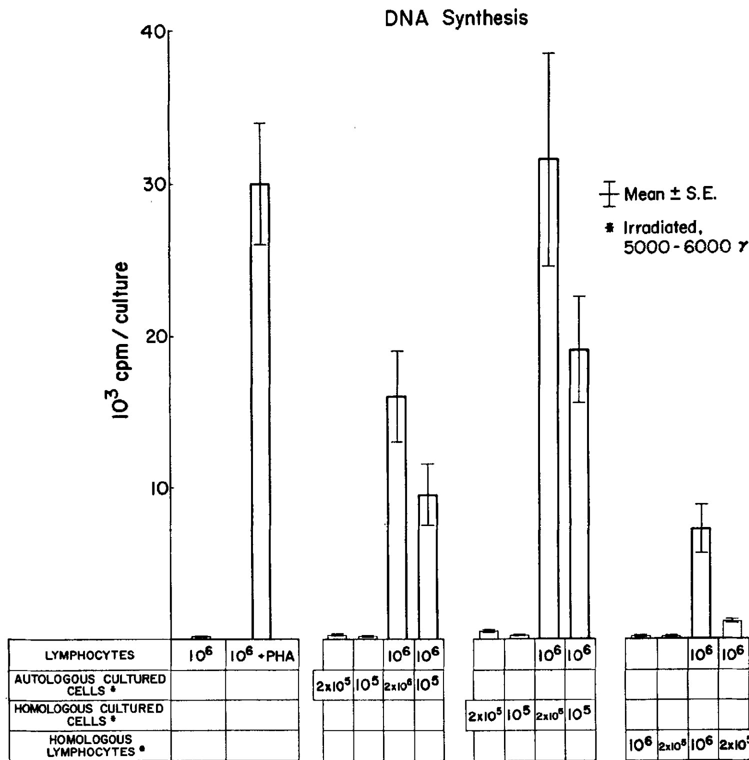

FIG. 1. *In vitro* lymphocyte response to PHA, homologous lymphocytes, and cultured lymphoid cells.

way reaction. Cultures were carried out in duplicate or triplicate. Each experiment included culture of lymphocytes in the presence of PHA, and appropriate controls (10^6 lymphocytes alone, in the same volume of culture medium and autologous plasma; irradiated target cells alone). The cultures were incubated at 37° for 3–4 days (PHA) or for 7 days (mixed-cell interaction). DNA synthesis was determined by the ^{3}H-thymidine incorporation method. 1.0 μCi of ^{3}H-thymidine (sp act 2.0 μCi/mmole, New England Nuclear Corporation) was added to each culture 4 hr before harvesting the cells.

Results and Discussion. Figure 1 shows the lymphocyte responses to PHA, autologous or homologous cultured cells, and homologous circulating lymphocytes. The stimulating effect of homologous cultured cells, at a ratio of 1:5 reactive cells, was comparable to that of PHA. Cultures at a 1:10 ratio, or a 1:1 ratio (not shown), gave less stimulation. Autologous cultured cells induced approximately half as great stimulation of thymidine uptake, on the average. Lymphocytes from each of the 10 subjects were stimulated by autologous cultured cells. Of interest was the fact that greater stimulation was seen with *autologous* cultured cells than with *homologous* circulating lymphocytes (the latter used at a 1:1 ratio, which is generally agreed to produce maximal stimulation).

To rule out the possibility that the stimulating effects of cultured cells might be related to some antigen adsorbed from the growth culture medium (especially, a component of calf serum) and not removed by washing with fresh plain medium, we performed four experiments which included an additional control system: 10^6 lymphocytes cultured with 2×10^5, or 10^5, irradiated autologous circulating lymphocytes which had been obtained previously and incubated for 1–3 days in culture medium containing 20% fetal calf serum. The incubated cells were washed three times in plain culture medium and re-

TABLE I. Lymphocyte Response to Autologous Circulating Lymphocytes, Cultured Lymphoid Cells, and Cell-Free Medium in Which Cultured Cells Were Suspended During Irradiation.

All target cells were irradiated with 5000–6000 R. Autologous lymphocytes were incubated in culture medium with 20% fetal calf serum for 1–3 days prior to experiments.

Lymphocyte donor	Additive	^{3}H-Thymidine incorporated (cpm)	
		With 10^6 lymphocytes	Target cells only
1. G. M., normal subject	None	126	
	PHA	15,600	
	Autologous lymphocytes, 2×10^5	289	126
	10^5	171	118
	Autologous cultured cells, 2×10^5	27,244	176
	10^5	12,180	82
2. Mar. C., normal subject	None	139	
	PHA	16,760	
	Autologous lymphocytes, 2×10^5	216	12
	10^5	86	14
	Autologous cultured cells, 2×10^5	14,434	81
	10^5	9716	11
3. B. H., Ca breast	None	57	
	PHA	23,782	
	Autologous lymphocytes, 2×10^5	26	12
	10^5	37	14
	Autologous cultured cells, 2×10^5	6078	69
	10^5	1575	29
4. Mat. C., malignant melanoma	None	195	
	PHA	18,107	
	Autologous lymphocytes, 2×10^5	117	4
	10^5	260	5
	Autologous cultured cells, 2×10^5	32,094	23
	10^5	26,602	72
5. C. H., normal subject	None	107	
	PHA	60,779	
	Autologous cultured cells, 2×10^5 in irradiation medium	9264	51
	Cell-free irradiation medium	170	
6. A. P., reticulum cell sarcoma	None	174	
	PHA	42,406	
	Autologous cultured cells, 2×10^5 in irradiation medium	17,000	602
	Cell-free irradiation medium	115	
7. J. M., normal subject	None	136	
	PHA	29,500	
	Cell-free irradiation medium (homologous cultured cells)	103	
8. P. G., normal subject	None	260	
	PHA	90,278	
	Cell-free irradiation medium (homologous cultured cells, line B.P.)	94	
	Cell-free irradiation medium (homologous cultured cells, line A.P.)	274	

TABLE II. Lymphocyte Response to Autologous Cultured Cells Grown in Medium Fetal Calf Serum or Medium with Autologous Serum.

The cell donor was B.P., a normal subject. Target cells were irradiated with 5000–6000 R.

Additive	^{3}H-Thymidine incorporation (cpm)	
	With 10^6 lymphocytes	Target cells only
None	67	
PHA	36,494	
Autologous cultured cells		
(autologous serum)		
2×10^5	21,418	363
10^5	19,230	298
(fetal calf serum)		
2×10^5	33,797	231
10^5	14,042	89

suspended in the medium prior to irradiation. In none of these experiments could a significant effect of incubated autologous circulating lymphocytes be detected. Additional controls included culture of lymphocytes in cell-free medium obtained from irradiated target cell suspensions by filtering the supernatant through a Millipore filter (0.22 μ), to exclude the possibility that a stimulant was produced during irradiation of cultured cells. These also gave negative results. In previous studies, we had shown that used growth culture medium contains no stimulants. This series of experiments is summarized in Table I.

Finally, to rule out the possibility that reactions might be due to a calf serum antigen incorporated only during *growth* of cultured cells, we performed an experiment with autologous cultured cells grown for 1 month in medium containing 10% autologous serum instead of fetal calf serum. The lymphocyte donor was a healthy individual; his cultured cells had a normal diploid karyotype and did not contain EB viral antigen. The results of this experiment are presented in Table II. There was little difference between the effects of cells grown in autologous serum and cells grown in fetal calf serum. We conclude that the stimulating effect of cultured cells cannot be attributed to any component of fetal calf serum.

The stimulation of lymphocytes by autologous cultured cells suggests that there is an antigenic disparity between circulating lymphocytes and cultured lymphoid cells from the same individual. It is possible that the surface antigens of lymphoid cells change during growth in an *in vitro* system. The greater stimulation observed with homologous cultured cells is consistent with this hypothesis; presumably, such cells would have both new antigen(s) attributable to *in vitro* growth and persistent HL-A antigens, resulting in a greater overall incompatibility with homologous circulating lymphocytes. It must be emphasized, however, that our results do not establish an immunologic basis for these mixed-cell reactions. It may well be that the effect of cultured cells is related to some component which stimulates small lymphocytes to enter a growth phase directly, rather than via the recognition of an antigenic difference.

Summary. Circulating lymphocytes from five normal individuals and from five patients with neoplastic disease were unequivocally stimulated by irradiated cultured lymphoid cells of lines established from the same donors. This reaction suggests that there is an antigenic disparity between these two types of cells. It is possible that the surface antigens of lymphoid cells may change during multiplication in an *in vitro* environment.

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