Brief Communication: Specific Colony Inhibition by Human Lymphoma Cells on an Established Human Lymphoma Cell Line [1]

B. Drewinko,[2] Giora Mavligit,[3] Ulo Ambus,[3] Carol Pritchett,[2] Jordan Gutterman,[3] *and* Evan Hersh [3]

SUMMARY—Freshly obtained human lymphoma cells were cocultivated with target cells derived from long-term cultures of human lymphoma, sarcoma, and melanoma cells and from Chinese hamster ovary. The fresh lymphoma cells inhibited colony formation of the lymphoma line but not of the other lines. We suggest that the lymphoma cells had the capacity of "autoaggressive" immunologic reactions.—J Natl Cancer Inst 51: 1377–1378, 1973.

COCULTIVATION of specifically sensitized lymphocytes with target tumor cells bearing the appropriate tumor-associated antigens may result in inhibition of the proliferative capacity of the target cells (*1*).

Specific reactivity against lymphoma target cells has been noted in circulating peripheral blood lymphocytes (PBL) from patients with lymphoma (*2*). This was presumed due to sensitization of the lymphocytes to a common lymphoma-associated antigen.

Fresh human neoplastic lymphocytes have not been used as effector cells, perhaps because these are not believed to be immunocompetent and therefore likely to possess a lethal potential of immunologic nature. This report describes a specific inhibition of colony growth induced in an established lymphoma cell line by fresh human lymphoid cells from a lymphomatous lymph node.

MATERIALS AND METHODS

For effector cells, a single cell suspension was prepared, as described in (*3*), from fresh tumorous lymph-node biopsy specimens obtained from 2 patients (C.B. and L.W.) with a histologic diagnosis of poorly differentiated lymphocytic lymphoma. Cell viability was determined by trypan blue exclusion. Differential counts on at least 1000 cells demonstrated that >95% were abnormal lymphocytes. This was determined by cytologic criteria (abundant cytoplasm, irregular nuclear configuration, smooth chromatin pattern, and the presence of a large nucleolus). Peripheral blood lymphocytes (PBL) were separated from defibrinated venous blood obtained from these patients and normal donors by differential flotation on Hypaque-Ficoll density solution (*4*). At least 90% of the cells had morphologic characteristics of mature lymphocytes. The rest were polymorphonuclear cells.

Established cell lines of human lymphoma (*5*), sarcoma (*6*), melanoma (*7*), and Chinese hamster ovary (*8*) were used as target cells. The lymphoma and sarcoma lines were grown in Ham's F-10 medium supplemented with fetal calf serum, vitamins, glutamine, and antibiotics. Both have been proved to carry common tumor-distinctive antigens specific for lymphoma and sarcoma, respectively (*9*, *10*). The other 2 cell lines were maintained in McCoy's 5A medium supplemented with fetal calf serum and antibiotics. Because of variations in plating efficiency (3.2% for the sarcoma line, 40% for human lymphoma and melanoma, and 70% for the Chinese hamster line) different densities of target cells were seeded in 60-mm petri dishes to yield 50–100 distinct colonies after 1–3 weeks of uninterrupted incubation. An array of 6 plating efficiency controls was prepared for each cell line. In addition to controls, effector cells (lymphoma cells and PBL) were added to triplicate dishes 24 hours after target cells were seeded in an effector:target cell ratio of 1000:1. After 24 hours of cocultivation at 37° C in a humid atmosphere of 5% CO_2 in air, the supernatant medium and nonadhering cells were removed. All dishes, including controls, were washed twice with Hanks' solution, and the remaining single target cells were allowed to grow into colonies in fresh medium. At the end of the appropriate period of incubation, dishes were rinsed with 0.9% NaCl solution and stained with 0.5% crystal violet in 95% ethanol. Colonies, defined as clumps of 50 cells or more, were counted under a stereomicroscope, and percent inhibition was calculated in reference to the plating efficiency controls for each cell line tested.

RESULTS

Tumorous lymph node cells and PBL from patients with poorly differentiated lymphoma significantly inhibited the colony growth of the established lymphoma line (table 1). PBL from 2 normal donors were significantly less inhibitory to the lymphoma target cells. Tumorous lymph node cells and PBL from patient L.W. were also incubated with the human sarcoma and melanoma and the Chinese hamster ovary lines. The inhibitory effect was minimal; this indicates a high degree of specificity ($P=0.05$) among the effector cells to a common lymphoma-associated antigen present on the established human lymphoma cell line.

DISCUSSION

Tumorous lymph node cells, derived from nodes in which the overwhelming population consisted of neoplastic lymphocytes, inhibited the colony-forming capacity of an established human lymphoma cell line. The inhibition was exerted in a target:effector cell

[1] Received June 13, 1973; accepted July 11, 1973.

[2] Department of Clinical Chemistry and Laboratory Medicine, The University of Texas at Houston M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77025.

[3] Department of Developmental Therapeutics, The University of Texas at Houston M. D. Anderson Hospital and Tumor Institute.

TABLE 1.—*Colony inhibition of established cell lines*

Patient	Effector cells	Established cell line	Percent inhibition ± SE
C.B	PBL	Lymphoma	70 ± 5. 3
C.B	Lymphoma cells.	Lymphoma	63 ± 13. 7
L.W	PBL	Lymphoma	78 ± 6. 0
L.W	PBL	Malignant melanoma.	7 ± 15. 3
L.W	Lymphoma cells.	Lymphoma	78 ± 6. 4
L.W	Lymphoma cells.	Chinese hamster ovary.	20 ± 11. 5
L.W	Lymphoma cells.	Malignant melanoma.	7 ± 17. 9
L.W	Lymphoma cells.	Neurogenic sarcoma.	2 ± 21. 3
A.F. (normal).	PBL	Lymphoma	11 ± 8. 7
A.F. (normal).	PBL	Malignant melanoma.	3 ± 9. 6
W.E.D. (normal)	PBL	Lymphoma	8 ± 12. 3

ratio similar to that obtained with PBL; no such effect was observed on either the human melanoma and sarcoma or the Chinese hamster cells. Such activity is clearly the result of a specific reaction between the effector cells (PBL and tumorous lymph node cells) and the target lymphoma cells; it is probably related to the tumor-associated antigen carried by the lymphoma cells. Therefore, neoplastic lymphocytes may possess a specific cytotoxic potential of "autoaggressive" immunologic nature.

REFERENCES

(*1*) HELLSTRÖM I, HELLSTRÖM KE, EVANS CA, et al: Serum mediated protection of neoplastic cells from inhibition by lymphocytes immune to their tumor specific antigen. Proc Natl Acad Sci USA 62:362–368, 1969

(*2*) SINKOVICS JG, DREYER DA, SHIRATO E, et al: Cytotoxic lymphocytes. I. Destruction of neoplastic cells by lymphocytes in cultures of human origin. Tex Rep Biol Med 29:227–242, 1971

(*3*) MAVLIGIT G, GUTTERMAN JU, MCBRIDE CM, et al: Multifaceted evaluation of human tumor immunity using a salt extracted colon carcinoma antigen (36650). Proc Soc Exp Biol Med 140:1240–1245, 1972

(*4*) THORSBY E, BRATLIE A: *In* Histocompatibility Testing, 4th Int Conf 1970, Baltimore, Williams & Wilkins, 1970

(*5*) TRUJILLO JM, DREWINKO B, AHEARN MJ: The ability of tumor cells of the lymphoreticular system to grow in vitro. Cancer Res 32:1057–1065, 1972

(*6*) TRUJILLO JM, DREWINKO B, LICHTIGER B, et al: Establishment in vitro of a human neurogenic sarcoma. Cancer Res 32:1066–1072, 1972

(*7*) BARRANCO SC, HO DW, DREWINKO B, et al: Differential sensitivities of human melanoma cells grown in vitro to arabinosylcytosine, Cancer Res 32:2733–2736, 1972

(*8*) BARRANCO SC, HUMPHREY RM: The effects of bleomycin on survival and cell progression in chinese hamster cells vitro. Cancer Res 31:1218–1223, 1971

(*9*) TRUJILLO JM, SHIVELY JA, List-YOUNG B, et al: Immunofluorescence studies of a lymphoid cell line derived from a patient with lymphocytic lymphoma. Tex Rep Biol Med 27:117–125, 1969

(*10*) DREWINKO B, MONTGOMERY CT, TRUJILLO JM: Demonstration of common sarcoma-associated antigen(s) in an established human neurogenic sarcoma cell line. Cancer Res 33:161–165, 1973