

**LYSOZYME SYNTHESIS BY ESTABLISHED HUMAN AND
MURINE HISTIOCYTIC LYMPHOMA CELL LINES***

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Macrophages and monocytes produce large amounts of lysozyme (LZM), most of which is secreted (1). Granulocytes also contain but do not normally secrete the enzyme (2). Elevated levels of LZM in urine and serum are a diagnostic indicator for acute monocytic leukemia and acute myelomonocytic leukemia (3). We report here an unusual human cell line, U-937, derived from a patient with histiocytic lymphoma¹ which synthesizes LZM. The cell line lacks immunoglobulin and Epstein-Barr virus (EBV) genome, but bears receptors for immunoglobulin and complement (C). For comparison we describe LZM synthesis by several murine histiocytic lymphoma cell lines with macrophage properties of antibody-dependent phagocytosis and exocytolysis (4, 5), and by other lymphomas in culture including a myelomonocytic leukemia resembling the progenitor of macrophages and granulocytes (6).

Materials and Methods

Human Cell Lines. The U-937 line was established from the pleural effusion of a patient with a generalized, diffuse histiocytic lymphoma as detailed elsewhere.¹ The cells bear receptors for immunoglobulin (31% EA) and C (11% EAC), phagocytose latex beads and formalin-fixed *Staphylococcus aureus* but not zymosan, and do not have detectable EBV genome or surface (fluorescence) or intracellular (gel diffusion) immunoglobulin. The line bears no resemblance to other EBV-positive or negative T- and B-lymphoid lines (7). Growth of U-937 is dependent on feeder layers or conditioned medium of human glia cells (8). Maximum cell density is 2×10^5 ml. Other human hematopoietic cell lines studied include B-lymphoblastoid cell lines of non-neoplastic origin and EBV-positive and negative neoplastic leukemia, lymphoma, and myeloma cell lines, according to the classification by Nilsson and Pontén (7). These lines were maintained in nonstirred suspension cultures fed twice a week by medium F10 or RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) supplemented by 10% newborn calf serum and antibiotics (100 IU/ml penicillin, 50 µg/ml streptomycin, and 1.25 µg/ml amphotericin B). All cultures were incubated at 37°C in a 5% CO₂-air atmosphere.

Murine Cell Lines. Cell lines were adapted from ascites or solid forms of lymphomas without the use of reducing agents, in some cases by repeated cycles of culture and mouse passages. Cell line PU5-1.8 was derived from lymphoma PU5-1 by transferring a solid tumor at passage 35 into mice intraperitoneally, transferring ascites cells three times, and adapting the ascites cells from passage 39 to culture. Another cell line, PU5-1/1, was derived from a tumor at animal passage 44, maintained in culture 1 mo, and then passaged once as ascites. Cell lines grow in RPMI 1640

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¹ Sundström, C., and K. Nilsson. 1976. Establishment and characterization of a human histiocytic lymphoma cell line. *Int. J. Cancer*. In press.

medium or Dulbecco's modified Eagle's medium containing 10% fetal calf serum in 10–13% CO₂ incubators using plastic Petri dishes (9), except for J774 and PC334 which were grown in plastic tissue culture dishes. Doubling times range from 10 to 24 h, except for PC334 which was slow growing. PC334 is strongly adherent (subcultured by trypsin-EDTA); PU5-1.8, J774, P388 D1, and WEHI-3 are loosely adherent; and the other lines nonadherent.

LZM. When cultures reached approximately 10⁶ cells/ml, but still in exponential growth with viability exceeding 85%, cells were harvested by centrifugation (1 min at 1,000 *g*). The cell pellet was lysed by 0.3 ml of 1% Nonidet P40 (Shell Chemical Corp., New York) in saline per 10⁷ cells for 5 min at 4°C. The extract and culture medium were centrifuged 10 min at 10,000 *g*. LZM was assayed in these supernates turbidimetrically (3) using egg-white LZM as a standard and results are stated in units of micrograms egg LZM. Cell lines U-937 and PC-334 do not reach a density of 10⁶/ml, and their activity has been normalized to 10⁶ cells. Serum, urine, ascites, and single cell suspensions from solid tumors were similarly assayed. Nonidet P40 did not affect the assay at the highest final concentrations used (0.2%).

Colony-Stimulating Factor (CSF). CSF was assayed as previously described (6).

Results

LZM Synthesis by Human Cell Lines. Only 1 (U-937) of the 18 hematopoietic cell lines tested synthesized detectable amounts of LZM (Table I). When U-937 cultures reach 10⁵ cells/ml, extracellular medium contains 0.5–0.8 μg LZM/ml, while intracellular enzyme levels amount to 0.3–0.8 μg/10⁵ cells. Thus 50 to 70% of the LZM synthesized appears to be secreted since there is negligible cell death under these conditions.

LZM Synthesis by Murine Cell Lines and Tumors. Cell lines J774 and PC334 from well-differentiated histiocytic lymphomas synthesize large amounts of LZM (Table II), 80–90% of which is secreted. Serum, urine, ascites fluid and intracellular content of ascites, and solid tumor cells of tumor-bearing mice contain greatly elevated levels of LZM. Another cell line, P388 D1, with macrophage properties (18) also produces similar amounts of LZM (not shown).² The WEHI-3 myelomonocytic leukemia line synthesizes moderate amounts of LZM, 50–70% of which is secreted. Mice bearing this tumor are also positive.

A spontaneous lymphoma PU5-1 with properties of B lymphocytes showed no LZM activity in tumors or in a cell line PU5-1/1. However, another line, PU5-1.8, produces easily detectable LZM, and the enzyme is associated with ascites and solid tumors formed from the latter cell line. An Abelson leukemia virus-induced lymphoma cell line, RAW 8, also produces LZM. Hematopoietic cell lines found negative for LZM include Abelson virus-induced lymphoma R8, myelomas, T lymphomas, mastocytoma, and chemically induced P388 lymphoma with lymphoid characteristics.

Synthesis of Colony-Stimulating Factor (CSF) by Cell Lines. CSF is required by bone marrow progenitor cells to proliferate into granulocytes and monocytes in vitro. Serum levels of CSF are elevated in mice carrying the myelomonocytic leukemia WEHI-3 (6). The cell line growing in culture since January, 1974, was therefore tested for CSF. When introduced into agar cultures at a final concentration of 1:10 (optimal conditions), WEHI-3 supernates stimulate the production of 100–150 colonies per 7.5 × 10⁴ mouse bone marrow cells. WEHI-3 CSF and normal mouse CSF are inactive with human progenitor cells. Neither human nor mouse CSF activity was detected in supernates of the other cell lines.

² Quincey, D., E. F. Osserman, and H. Koren. Manuscript in preparation.

TABLE I
Lysozyme Associated with Human Cell Lines

Positive cell line	Medium	Intracellular
	$\mu\text{g/ml}$	$\mu\text{g}/10^6$
Histiocytic lymphoma U-937 (footnote 1)	5-8	3-8
Negative cell lines		
B lymphoblastoid EBV ⁺		Myeloma
U-704 (10)		266BL (8, 10)
WIL-2 (11)		8226 (7, 10, 11)
8866 (11)		T lymphoblastic leukemia
UM-37 (11)		MOLT 4 (7, 10)
Burkitt's lymphoma EBV ⁺		CCRF-CEM (15, 16)
RAJI (10, 11, 12)		CCRF-H-SB-2 (15, 16)
DAUD1 (10, 11, 12)		CML
RAMOS (12)		K562 (17)
Non-Burkitt's lymphoma EBV		Bone marrow, epitheloid
U-698 (10, 13) -		D98 (16)
U-715 (10, 13) -		J111 (16)
S-95 (10, 14) +		

Lower limit of LZM detection was 0.2 $\mu\text{g/ml}$ in the extracellular medium per 10^6 cells and 0.05 $\mu\text{g}/10^6$ cells in lysates. LZM was not found in control glial cells or culture supernates used as feeder layers for U-937 growth. Reference to cell lines are given in parentheses.

Discussion

The group of histiocytic lymphomas comprises a variety of histopathologic types making diagnosis and classification difficult. In the mouse, there are transplantable tumor models for: (a) the well-differentiated histiocytic lymphoma with properties of macrophages (4), and (b) the pleomorphic reticulum cell sarcomas occurring spontaneously in SJL mice, which in some respects resemble Hodgkin's disease by presenting a variety of cell types (21). The well-differentiated histiocytic lymphomas J774 and PC334 exhibit high levels of phagocytosis (latex beads and zymosan), pinocytosis (neutral red), and antibody-dependent phagocytosis and exocytolysis of erythrocytes (5) not found with spontaneous SJL tumors (unpublished observation). Similar macrophage-like properties have been described for cell lines P388 D1 (18) and 1C-21 (22). Tumor forms of the latter two lines are not available.

We show here that high levels of lysozyme are found in mice bearing well-differentiated histiocytic lymphomas, as described by Riblet and Herzenberg (23), and that cell lines derived from these tumors maintain LZM synthesis in vitro. The culture macrophage line 1C-21 was previously shown to synthesize LZM (24). Some Abelson virus-induced neoplasms are thought to be of immature myelomonocytic origin (25), confirmed by LZM synthesis in the RAW 8 cell line derived by infecting spleen cells in vitro (19). The relation of LZM-positive PU5-1.8 line to the lymphoma PU5-1 remains unclear, but it is not a contaminant of a different cell line since no other line exists with PU5-1.8 properties (9).

Besides lysozyme synthesis, another characteristic of the histiocyte-monocyte series is the production of CSF (26). Only the LZM⁺ myelomonocytic leukemia WEHI-3 was found to synthesize CSF. The CSF⁻ LZM⁺ lines PU5-1.8 and RAW 8 may represent an earlier form of this cell type unable to differentiate further.

Rat myelogenous leukemia and chloroma cell lines have been reported positive by histochemical staining for LZM (27). Of the human lines tested, only U-937 histiocytic lymphoma and a preliminary report (28) of a myelomonocytic

TABLE II
Lysozyme Associated with Murine Tumors and Cell Lines

Cell line	Strain	Tumors				Cell cultures	
		Serum	Urine	Ascitic Fluid	Cells	Medium	Cells
		$\mu\text{g/ml}$	$\mu\text{g/ml}$	$\mu\text{g/ml}$	$\mu\text{g}/10^6$	$\mu\text{g/ml}$	$\mu\text{g}/10^6$
Histiocytic lymphoma							
J774 (4, 5)	C*	5-200	200-700	40-1500	0.04-0.22	2-25	0.02-0.5
PC334 (R. Riblet)	C x NZB	ND	ND	45-150	ND	10-100	ND
Abelson lymphoma							
RAW 8 (9, 11, 19)	C	3-14	3-63	ND	ND	2-5	0.1-0.5
R8 (9, 19)	C x B	2-3	2-7	2-11	<0.007	<0.3	<0.007
Myelomonocytic leukemia							
WEHI-3 (6, 20)	C	10-17	16-300	50-200	0.1-1.0	0.1-0.8	0.05-0.8
Spontaneous lymphoma							
PU5-1/1 (11)	C	2-8	3-8	7-25	<0.02	<0.1	<0.01
PU5-1.8 (9, 11)	C	8-15	50-500	120-280	0.3-0.7	1-50	0.2-10
Myelomas							
MOPC-315, P3 (11)	C	1-3	0.9	1-3	<0.02	<0.1	<0.001
T lymphomas							
EL4, S49 (11)	B, C	ND	ND	ND	ND	<0.1	<0.001
Mastocytoma							
P815 (11)	D	ND	ND	1-3	ND	<0.1	<0.001
Lymphoid neoplasm							
P388 (9, 18)	D	ND	ND	ND	ND	<0.1	<0.01

* Strains of mice: C, BALB/c; B, C57BL/6; D, DBA/2.

leukemia line synthesize LZM. These are the first examples of a human neoplasm adapted to culture and positively typed by a functional assay for the monocyte-granulocyte cell series. Philadelphia chromosome has been described in the myeloid line K562 (17), although the subline studied here lacks the karyotypic marker.³ Three cell lines of Epstein and Kaplan (29) from diffuse histiocytic lymphoma patients resemble the original tumor cells morphologically and show staining characteristics compatible with a histiocyte origin. LZM production was not studied.

In view of the variety of aberrant behavior shown by many culture lines, further studies with the cell lines described here are necessary to confirm their retention of myelocyte/monocyte-specific properties. The lines should aid in diagnosis and classification of lymphomas, analysis of mechanisms of drug action and evaluation of new chemotherapeutic agents, and investigations of tumor antigens as well as of the normal functions of the monocyte, histiocyte, and granulocyte series of cells.

Summary

A human cell line established in culture from a histiocytic lymphoma patient synthesizes and secretes the monocyte-granulocyte specific enzyme lysozyme. 18 other human cell lines with characteristics of T-lymphocyte, B-lymphocyte, Burkitt's lymphoma, non-Burkitt's lymphoma, myeloma, and bone marrow epithelial cells were not associated with lysozyme.

Among murine cell lines, lysozyme was produced by (a) three histiocytic

³ Klein, E., H. Ben Bassat, H. Neumann, P. Ralph, A. Polliak, and F. Vanky. 1976. Characterization of the K562 cell line derived from a chronic myeloid leukemia patient. Manuscript submitted for publication.

lymphoma or macrophage lines, which mediate antibody-dependent phagocytosis and cytolysis; (b) myelomonocytic leukemia line which also secretes myeloid colony-stimulating factor; and (c) a spontaneous lymphoma and an Abelson leukemia virus-induced lymphoma. Lysozyme-negative lines include another Abelson lymphoma, myelomas, T lymphomas, and mastocytoma.

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