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Enhanced Establishment of Lymphoblastoid Cell Lines With the Ficoll-Hypaque Density Gradient Technique (38399)

Comparison of Cell Lines from Patients With Systemic Lupus Erythematosus, Infectious Mononucleosis and Normal Controls[1]

ALAN L. BRODSKY AND ERIC R. HURD[2]
(Introduced by M. Ziff)

The Rheumatic Diseases Unit, Department of Internal Medicine, The University of Texas Health Science Center, Dallas, Texas 75235, and the Dallas VA Hospital, Dallas, Texas 75216

While the precise etiology and pathogenesis of systemic lupus erythematosus (SLE) remain largely speculative at the present time, much of the current research interest is centered about the lymphocyte and its relationship to alterations in both humoral and cellular immunity. The establishment of long-term lymphoblastoid cell lines from human peripheral blood is a well-described technique (1–3) which seems uniquely suited to the study of lymphocytes from patients with SLE, since such lines provide a readily available source of large quantities of actively metabolizing cells which might retain some of the abnormal properties of the donor. Accordingly, we attempted to establish and study lymphoblastoid cell lines from patients with SLE. For comparison, lines were also established from patients acutely ill with infectious mononucleosis (IM), as well as from healthy volunteers matched for age, race and sex.

Since most studies have demonstrated that the cells of origin for such cultures are of the lymphoid series (4, 5), it seemed logical to begin with a pure mononuclear cell preparation in hopes of increasing the ease of establishment of such lymphoblastoid cell lines using the Ficoll–Hypaque density gradient technique.

Methods. Patients. Ten patients with the diagnosis of SLE being followed in the Parkland Memorial Hospital (PMH) Outpatient Rheumatology Clinic or as in-patients being seen in consultation by the Rheumatology Service were randomly selected. Patients ranged in age from 20 to 55 years (mean 30.6). There were seven blacks, two whites and one Latin-American. All were female. All were being treated with prednisone in doses ranging from 7.5 to 100 mg per day, but none were on cytotoxic agents. A control group matched for age, race and sex was selected from healthy hospital and medical school employees. Eight patients with acute IM were also selected (mean age 18.2 years, five females, three males, seven whites, one black). Because of the anticipated difficulty in establishing cultures from normal persons, another smaller group of healthy adult medical school or hospital employees with a well-documented previous history of infectious mononucleosis similarly volunteered to donate blood samples.

Culture techniques. A modification of the method described by Glade and Broder (3) was used. Twenty-five cubic centimeters of heparinized blood was obtained from each subject. Ten cubic centimeters were allowed to sediment at 37° for approximately 2 hr, and the plasma and buffy coat were then separated and spun at 800 rpm × 30 min. The pellet was suspended in supplemented RPMI-1640 (including 20% fetal calf serum, L-glutamine and penicillin–streptomycin) and placed in 25 cm^2 tissue culture flasks at a final concentration of one to three million cells/cc. Cultures were incubated at 37° in a 5% CO_2 atmosphere. Mononuclear cells were separated from the remaining 15

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[2] Address requests for reprints to Dr. Eric R. Hurd, Department of Internal Medicine, The University of Texas Health Science Center, 5323 Harry Hines Blvd., Dallas, TX 75235.

TABLE I. Comparison of Ficoll-Hypague and Gravity Sedimentation Techniques for Establishment of Lymphoblastoid Cell Lines.

Study group	Method[a]	Pos	Neg	Total	% Pos	*P*
SLE	F/H	7	3	10	70	
	SED	5	5	10	50	
Infectious mononucleosis (IM)	F/H	7	1	8	87.5	
	SED	7	1	8	87.5	
Normal (ARSM)[b]	F/H	8	2	10	80	
	SED	5	5	10	50	
Normal (Previous IM)[c]	F/H	2	2	4	50	
	SED	0	4	4	0	
Normal (PHA)[d]	F/H	3	2	5	60	
	SED	1	4	5	20	
Normal total (ARSM + Prev IM + PHA)	F/H	13	6	19	68.4	0.025
	SED	6	13	19	31.6	
TOTAL	F/H	27	10	37	73.0	0.029
	SED	18	19	37	48.6	

[a] F/H = Ficoll-Hypaque density gradient; SED = Gravity sedimentation.
[b] ARSM = Healthy adults matched with SLE patients for age, race and sex.
[c] Healthy adults with previous history of IM.
[d] Healthy adults with previous history of IM with 0.025 μg/ml of PHA added to cultures.

cc of fresh heparinized blood by density gradient centrifugation using the Ficoll–Hypaque (F/H) technique (7). This gave a separation containing approximately 98% mononuclear cells. The cells were washed three times in RPMI-1640 and then suspended at a concentration of one to three million cells /cm^3 and incubated as described above for the gravity sedimented leukocytes. To the leukocyte suspensions from five normal controls, 0.025 μg/ml of highly purified PHA[3], a submitogenic dose (3), was added in hopes of enhancing ease of establishment.

Cultures were fed weekly with supplemented RPMI and observed for lymphoblastoid transformation. Subcultures were subsequently established, and aliquots frozen at −180° F in 10% DMSO for future analysis.

Statistical calculations. The Mann–Whitney U Test (8) was used for computer programming to determine significant differences between the various culture groups.

Results. A. Comparison of F/H and sedimentation techniques in establishment of lymphoblastoid cell lines (Table I): While the numbers in each individual group were too small for statistical analysis, there appeared to be a noteworthy difference between the sedimentation and F/H methods in the control groups. Cultures from volunteers who had had previous IM, or to which "submitogenic" doses of PHA had been added, had no apparent advantage over those from healthy normal controls. The differences between the density gradient and sedimentation methods were statistically significant for the control cultures when grouped together ($P = 0.025$), and also for the total group of cultures ($P = 0.029$).

B. Comparison of time of establishment for cultures from patients with SLE, IM, and control groups (Fig. 1, Table II):

There was no apparent difference between the sedimentation and F/H methods with regard to time of establishment of cultures. However, there was a clear difference in the time of lymphoblastic transformation with IM cultures undergoing transformation first, followed by the SLE cultures, and then the normal controls. The

[3] Phytohemagglutinin #MR68, Wellcome Research Laboratories, Bechenham, England.

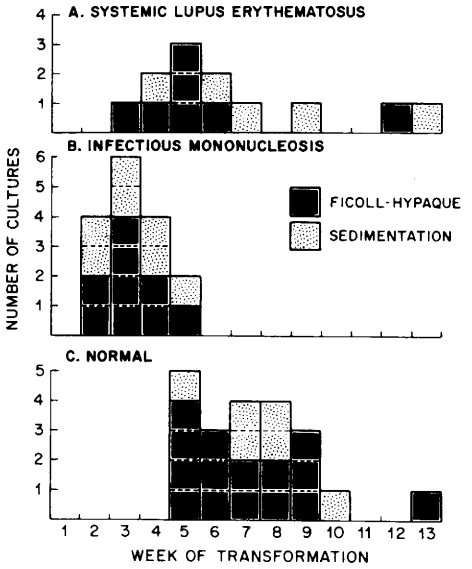

FIG. 1. Week of lymphoblastic transformation for lymphoblastoid cell lines showing comparison of Ficoll–Hypaque density gradient and sedimentation techniques for separation of white blood cells.

mean week of blastic transformation was significantly earlier for the IM group than for the normals ($P < 0.01$) or the SLE group ($P < 0.01$). The difference between the SLE and normal groups was not statistically significant.

Discussion. It is generally presumed that the mononuclear cells of peripheral blood are the precursors which ultimately undergo lymphoblastic transformation (1–5). Although many of the specific details of the micro-environment necessary for lymphoblastic transformation to occur have yet to be fully delineated, it is known that there are critical concentration limits for viable cells in evolving cultures (3). Using F/H density gradient centrifugation to give an almost pure population of mononuclear cells appeared to be a logical approach to increase the yield of positive cultures. This indeed turned out to be the case, particularly for cultures obtained from healthy persons, with the percent of positives increasing from 31.6% to 68.4%. Our baseline rate of success was almost identical to that reported in the literature (6). Our Ficoll–Hypaque gradients contained essentially pure mononuclear cells including lymphocytes and monocytes, both of which have been shown to be necessary for blastogenesis and DNA synthesis in human leukocyte cultures (9–12).

TABLE II. Week of Lymphoblastic Transformation.

Patients	Method[a]	Mean (wk)
	F/H	5.7
SLE	SED	7.8
	Total	6.6
Infectious	F/H	3.2
mononucleosis	SED	3.3
(IM)	Total	3.2
Normal	F/H	7.1
(ARSM + Prev IM + PHA)	SED	7.5
	Total	7.3

[a] F/H = Ficoll-Hypaque density gradient; SED = Gravity sedimentation.

While three SLE cultures were established earlier than any of the normal controls, the average week of transformation for the SLE group was not significantly different from the normal group. Thus, although the pathogenesis of SLE may involve a lymphocyte which has been altered or activated by an infectious agent, the present study fails to demonstrate any outstanding abnormalities with respect to the ability of SLE lymphocytes to establish long-term suspension cultures.

Summary. Lymphoblastoid cell lines were established using peripheral blood which was obtained from ten patients with systemic lupus erythematosus, eight patients with infectious mononucleosis and 19 normal individuals. In each instance, the blood was divided into two portions. One portion was prepared for culture by gravity sedimentation of the white blood cells. The other portion was prepared by use of the Ficoll–Hypaque density gradient technique for separation of pure mononuclear cells. Results demonstrated that the Ficoll–Hypaque technique was significantly more efficient for establishment of lymphoid suspension cultures than was the gravity sedimentation method.

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