Lymphoblastoid Cell Cultures from Patients with Infectious Hepatitis[1] (34363)

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Continuous lymphoblastoid cell lines have been established from lymphoid tissue and peripheral blood obtained from patients with various neoplastic (1–3) and infectious diseases (4–6) as well as from apparently normal donors (7, 8). Cell lines are particularly easily developed from the peripheral blood of patients with infectious mononucleosis, and it has been suggested that this may reflect the presence of an associated infectious agent (9).

In October, 1968, an outbreak of infectious hepatitis (IH) occurred among military personnel stationed at Fort Belvoir, Virginia, and peripheral blood was obtained from 35 patients with diagnosed acute IH. It was found that continuous lymphoblastoid cell cultures were more readily established from the peripheral blood of these IH patients than from the blood of healthy laboratory personnel who served as controls.

Material and Methods. Hepatitis patients. Thirty-five young males (18–36 years old) with IH ranging from subclinical anicteric disease to severe debilitating illness with jaundice were studied. These individuals were part of an outbreak which had clinical and epidemiologic features generally considered typical of infectious hepatitis, although laboratory studies were not carried out to exclude viral hepatitis of other types. No inoculations, dental work, administration of blood products or use of narcotics could be implicated in this epidemic which involved over 250 men confined to a single military base. All of the donors had persistently elevated serum glutamic pyruvic transaminase (SGPT) levels but negative heterophile tests (10) for infectious mononucleosis. Liver biopsy which was performed on 31 patients supported the diagnosis of IH in most cases. They were confined at DeWitt Army Hospital during the course of their illness and specimens of blood for leukocyte culture were obtained within 3–14 days of diagnosed onset of illness.

Healthy controls. Peripheral blood was obtained from 16 healthy individuals (11 males and 5 females) ranging from 21 to 42 years of age. All were personnel of this laboratory and none had a history of hepatitis within the last 5 years.

Preparation of cultures. Cultures were prepared using an adaptation of the method of Gerber (8). Leukocytes were separated from 30 ml of fresh heparinized peripheral venous blood by gravity sedimentation for 60 min at 37° in 40-ml conical centrifuge tubes. The plasma supernatant containing nearly all of the leukocytes, was removed and centrifuged at 1000 rpm for 10 min. The sedimented leukocytes were washed twice in culture medium consisting of RPMI 1640 (7) containing 20% fetal bovine serum; glutamine, 2 m*M*; penicillin, 200 μ/ml; and streptomycin, 200 μg/ml. The cells were resuspended in culture medium at a concentration of 5×10^6 cells/ml in 250-ml Falcon plastic tissue culture flasks and incubated stationary at 37° in a humidified atmosphere of 5% CO_2 in air. They were fed twice weekly by centrifuging the entire culture and resuspending in fresh medium. As the number of viable cells in the culture decreased, as determined by trypan blue dye exclusion, the volume of medium was reduced to maintain a viable cell population of greater than 5×10^5 cells/ml, until a volume of 2 ml was

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reached. Cultures were maintained at that volume until cell proliferation developed or until no viable cells could be found by observation in a hemocytometer counting chamber.

Results. Continuous suspension cell lines were established from 21 of 35 (60%) IH specimens and 3 of 16 (19%) normal donors. This difference is significant at the level of $0.01 < p < 0.05$. There was no apparent difference between the clinical or laboratory findings or the severity of changes observed on liver biopsy of the IH patients from whom continuous cultures were established and those whose leukocytes did not proliferate (Table I).

The time at which cultures became established was generally earlier for cultures derived from patients with IH than those derived from normal donors. The onset of rapid

TABLE I. Establishment of Cultures from Patients with Acute Infectious Hepatitis.

Patient	Highest SGPT[a]	Duration of elevation of SGPT (days)	Jaundice[b]	Lymphoblastoid culture developed	Culture designation
B. Al.	640	>104	—	+	BS-HLH-9[c]
T. Ba.	53	25	—	—	
H. Br.	160	43	—	+	BS-HLH-4
R. Br.	230	99	—	—	
R. Bu.	122	>104	—	+	BS-HLH-21
R. Bx.	340	36	—	+	BS-HLH-6
A. Ca.	>800	50	+	+	BS-HLH-15
L. Ca.	>130	25	—	+	BS-HLH-18
J. Ch.	56	18	—	+	BS-HLH-14
H. Co.	297	>104	—	+	BS-HLH-23
J. Do.	62	54	—	+	BS-HLH-8
M. Du.	>800	65	+	—	
R. Ev.	100	32	—	+	BS-HLH-20
G. Ge.	88	48	—	+	BS-HLH-25
D. Gr.	>1000	50	+	—	
P. Gr.	>125	59	—	—	
G. Ha.	>800	>106	+	—	
J. Je.	>1250	44	+	—	
S. Kn.	200	>105	—	—	
A. La.	192	>105	—	+	BS-HLH-16
J. Le.	91	>104	—	+	BS-HLH-13
L. Ma.	>1250	34	+	+	BS-HLH-17
T. Ma.	160	98	—	+	BS-HLH-3
R. Mo.	92	40	—	+	BS-HLH-11
D. Mo.	52	27	—	—	
D. Ni.	370	60	—	—	
T. No.	64	41	—	—	
B. Po.	>800	>104	+	+	BS-HLH-24
J. Sh.	70	18	—	+	BS-HLH-19
W. Sl.	>1250	62	+	+	BS-HLH-10
A. Sm.	>800	41	—	—	
C. Sm.	>200	18	—	+	BS-HLH-12
D. Sw.	58	22	—	—	
A. Wh.	60	41	—	—	
J. Wi.	180	49	—	+	BS-HLH-7

[a] Normal range: mean ± SD = 29 units/ml ± 20.

[b] Jaundice defined as total bilirubin greater than 2 mg/100 ml.

[c] Biologics Standards, human lymphoblastoid, hepatitis.

TABLE II. Onset of Continuous Growth of Leukocyte Cultures from Patients with Infectious Hepatitis and Control Donors.

Culture donor	Incubated before development of continuous growth[a]					
(days):	20–29	30–39	40–49	50–59	60–69	70–79
Infectious hepatitis (21/35)[b]	4	8	7	1	0	1
Healthy control (3/16)	0	0	0	2	0	1

[a] Mean time at which continuous growth began was 40 days for IH cultures and 60 days for controls.

[b] Twenty-one cultures established from 35 specimens studied.

growth in the 3 control cultures that became established occurred on days 56, 56, and 70 while only 2 of the IH cultures required 56 days or longer to become established (Table II).

All established cultures were tested for the presence of bacteria, fungi, and mycoplasma with negative results. Preliminary studies failed to reveal the presence of viruses that would be recognized by conventional techniques for isolation of cytopathic, hemadsorbing, or interfering agents. Detailed results of these and other virologic studies which are in progress will be reported later.

The morphology and growth characteristics of these lines are similar to those previously reported for cell lines established from other diseases. The cells grow in suspension, usually in clumps, and the viable cell count doubles every 24–48 hr under optimal culture conditions. Light microscopic examination of Giemsa stained cells revealed the majority of cells to be lymphoblasts; the remainder resemble atypical and mature lymphocytes (Fig. 1).

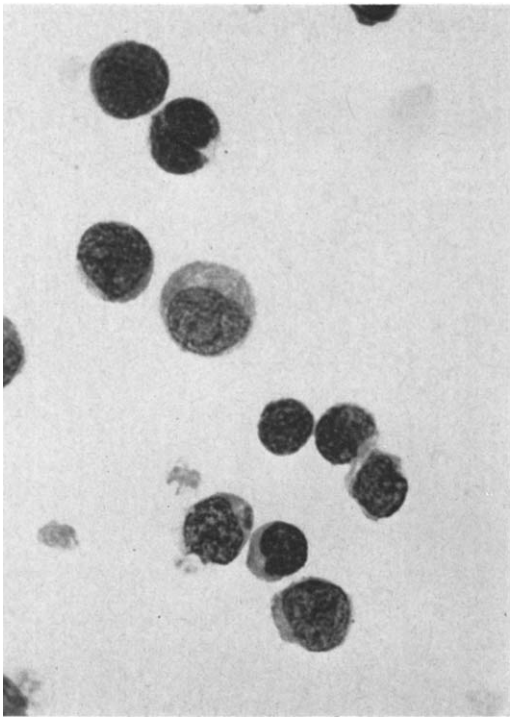

FIG. 1. Typical morphology of BS-HLH-10 lymphoblastoid cells is demonstrated; giemsa stain; ×1000.

Discussion. The proliferative tendency of lymphoblastoid cells after prolonged incubation as observed here contrasts with the mitotic suppression described by Mella and Lang (11) in short-term culture of fresh lymphocytes from patients with IH. However, Willems *et al.* (12) demonstrated an absence of this effect after 6 days of incubation of leukocytes from patients with viral hepatitis. Thus, this disparity may be explained by the major differences in cultivation techniques, the most notable of which is the prolonged cultivation time employed by us. It is also possible that cells which ultimately proliferate into continuous cultures are different from those leukocytes in which mitotic suppression has been observed in short-term studies.

The precise cell of origin of these and other continuous cell lines derived from peripheral blood leukocytes has not been defined. However, previous reports of morphology (13), globulin production (14) and phagocytic characteristics (15) suggest that cells of the lymphocyte and/or monocyte series are involved.

Reports of attempts to cultivate cell lines from the peripheral blood of normal donors indicate that methods which employ larger starting volumes of donor blood, *i.e.*, 500 ml (8), yield a considerably higher percentage of successful continuous cultures than attempts made with smaller starting volumes (4, 5) such as that employed in our method (30 ml). It is possible that circulating leukocytes possessing the capacity for proliferation in culture are present in normal individuals but at a low concentration.

Among the possible explanations for the proliferative phenomenon observed in leukocytes obtained from IH patients are: (i) it is an *in vitro* manifestation of the host's response to antigenic stimulation which occurred *in vivo*, and (ii) it is a direct effect caused by infection of the donor lymphocytes with a specific viral agent.

Wood and Frenkel (16) demonstrated increased incorporation of DNA precursors into peripheral lymphocytes *in vitro* and increased numbers of atypical lymphocytes *in vivo* in diseases in which increased immunologic activity probably was present. These findings were made in patients with pemphigus vulgaris, chronic ulcerative colitis, and several drug hypersensitivity conditions. They suggest that a proliferative tendency in peripheral lymphocytes in some instances may be a feature of the immunologic response to stimulation by a variety of antigens.

There is evidence, however, that direct infection of lymphocytes by at least one viral agent, Epstein-Barr virus (EBV), may stimulate proliferation of lymphoid cells in tissue culture. This herpes-like agent has been found in lymphoblastoid cells cultures from normal donors (7, 8) as well as from patients with a variety of diseases (9, 13) and has been incriminated by sero-epidemiologic evidence as the probable etiologic agent of infectious mononucleosis (17). A recent report described the establishment of lymphoblastoid cultures from 3 patients with serum hepatitis (6). While all 3 of the culture lines contained viral particles resembling EBV by electron microscopy and 2 were positive in fluorescent antibody studies, it seems unlikely that EBV plays any other than a coincidental role in viral hepatitis. Its size and lability are different from the assumed properties of the agents of infectious and serum hepatitis (18) and anti-EBV antibody titer changes have not been observed in prospectively studied sera from hepatitis cases (17).

Work is currently in progress to seek differences between the cell lines derived from healthy controls and those from patients with IH which might account for the ease of establishment of continuous cultures from leukocytes from patients with IH. Such differences might provide clues to the presence and nature of the etiologic agent of this disease.

Summary. Continuous suspension cultures were more readily established from circulating leukocytes obtained from patients with infectious hepatitis than from those from healty controls. Cultures were established from 21 of 35 (60%) patients from a single epidemic of infectious hepatitis and 3 of 16 normal donors. Generally, cultures became established earlier from the donors with hepatitis than from the controls. This proliferative tendency did not correlate with severity of disease in the patients.

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