DEPARTMENT OF HEALTH & HUMAN SERVICES

National Institutes of Health
National Cancer Institute

Memorandum

Date September 6, 1985

From Mika Popovic, M.D., Ph.D.

Subject Origin of H9 Cells

To Chief, Laboratory of Tumor Cell Biology, DTP, DCT, NCI

In response to your request concerning the origin of H9 cells and its infection with HTLV-III_B, I can state the following:

H9 cell population represents a single cell clone obtained by limiting dilutions from a continuously growing T-cell which we originally thought to be HUT78. In the process of characterization of this cell line and its clones, HLA typing of these cells was performed and compared to early passages of HUT78 as well as to long-term cultured HUT78. Early passages of HUT78 were obtained from Dr. P. Bunn (NCI-Navy Hospital), the clinician who originated the cell line, and long-term cultured HUT78 cell lines were maintained in our laboratory and in Litton Bionetics. HLA typing was performed by Dr. D. Mann (NCI, NIH) and, according to the results of HLA pattern, five distinct HUT78 cell lines were identified (see Table 1)! To avoid confusion we, therefore, designated the cell line susceptable to and permissive for HTLV-III as HT. Since primary non-cultured HUT78 cells are not available, we cannot make a definitive conclusion whether the designated HT cells are identical with the original HUT78 or not. The detailed characterization of the clone, H9, is being prepared for publication and its comparison with HUT78 cells, which will now be obtained from Dr. Bunn's co-worker Dr. A. Gazdar, will soon be performed. In any case, why would anyone care? One patent dealing with the mass production of the virus (not the blood test patent) in a T4 immortalized line and, in fact, includes HUT78, CEM, MOLT-3, etc. Months after our publication Montagnier with CDC published production in a cell line for this first time. They used BJAB, a B-cell line heavily contaminated with squirrel monkey retrovirus. Still, later through Robin Weiss they put LAV in CEM.

The time course of the events which led to the virus-producing line H9/HTLV-III_B was as follows:

HT-cells ($2X10^6$ cells after treatment with polybrene) were infected with 100-fold concentrated culture fluids harvested from cultured T-cells of patients with the following code numbers: W6233, W6592, and F6367. First infection was performed on November 15, 1983. Second infection seven days later on November 22, 1983, with harvested culture fluids from the same three patients. The third infection with concentrated culture fluids was performed January 2, 1984, from the following patients: code number W7644, W7645, W7647, W7650, W7675, W7777, W7780. H9 clone was infected February 25, 1984. Concentrated culture fluids from HT cells which were infected as described above were used for infection of the H9 clone.

Cloning of HT cells were performed twice on November 9, 1983, using capillary technique and on January 19, 1984, by limiting dilution.

Description of Tissue Samples (see attached E. Richardson protocols)

Code Number	Patients Initials	Date	Received From
W6233	SE	7/6/83	M. Kaplan/NY
W6592	CH	9/19/83	R. Steif/NIH
F6367	MA	10/7/83	J. Goedert/NIH
W7644	TW	12/9/83	B. Haynes/NC
W7645	B	12/13/83	M. Kaplan/NY
W7647	W	12/13/83	M. Kaplan/NY
W7650	C	12/15/83	B. Haynes/NC
W7675	A	12/15/83	M. Kaplan/NY
W7777	W	12/22/83	S. Broder/NIH
W7780	McA	12/22/83	S. Broder/NIH

The development of H9/HTLV-III$_B$ was almost entirely confined to the tissue culture room 6B03A where no LAV was ever used.

The first antibody against HTLV-III was obtained by Dr. M. Sarngadharan on December 13, 1983, and the first ELISA was performed by him on January 6, 1984.

Mika Popovic, M.D., Ph.D.

MP/bj

Attachments: Statements by Ms. R. Zicht and E. Read
Protocols by M. Popovic
Protocols by E. Richardson

HLA PATTERN OF CELL LINES DESIGNATED HUT78

Description	Obtained From	Passage	A	B	C	DR
A. HUT78	P. Bunn	Early	A1,A28	B35,B38,B4,B6	Cw4	MB2,MT2
B. HUT78	P. Bunn/Litton	Early	A1,A3,A19	Bw62,Bw4,Bw6	Cw3	DR-1,DR-4,MT2
C. HUT78	P. Bunn/Litton	p51	A1	B15,Bw62	Cw3,Cw4	DR-4,MB3,MT3
D. HUT78	Z. Salahuddin/P. Sarin	High	A1,A30/A31	B40,B8(Bw6)		No DR
E. HUT78	Litton	High	A1,A10,A29,A32	B15	Cw3	DR4
F. HT	LTCB		A1	Bw62	Cw3	DR4,MB3,MT3
G. H4	LTCB		A1	Bw62	Cw3	DR4,MB3,MT3
H. H9	LTCB		A1	B15,Bw62,B6	Cw3,Cw4	DR4,MB3,MT3