DEPARTMENT OF HEALTH & HUMAN SERVICES

National Institutes of Health
National Cancer Institute

Memorandum

Date November 28, 1984

From Visiting Associate, LTCB, DTP, DCT, NCI

Subject Documentation of Chronological Detection and Isolation of HTLV-III$_{RF}$

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

I am writing this MEMO to describe and document the chronology of detection and isolation of HTLV-III$_{RF}$, the isolate which is called "Haitian" in the Laboratory of Tumor Cell Biology. I wish to explain the reasons why this, one of the first well documented and carefully followed isolate (kept in a separate laboratory and later in a separate incubator, etc.), which had been obtained and propagated in HT cells almost one year ago, has not yet been selected for large scale production and distribution as a prototype of HTLV-III.

A total of two frozen ampules of separated viable lymphocytes were obtained on October 18, 1983 from Dr. James Hoxie (Department of Medicine, University of Pennsylvania, Philadelphia, PA [telephone (215) 662-3910]). Patient's serum was provided to Dr. Robert-Guroff (LTCB, DTP, DCT, NCI, NIH).

Patient: Roland Ferdinand, 25 year old, Hispanic from Haiti, heterosexual, diagnosed as diffuse lymphadenopathy and oral monilia, died January 1984.

Serum from patient RF is available in both laboratories (LTCB at NCI and at the Department of Medicine, University of Pennsylvania in Philadelphia). One ampule of primary non-cultured mononuclear cells from patient RF is stored at LTCB. The cultures of RF cells (code No. W7152) were set up on November 15, 1983 according to well established procedures (TCGF, etc.) and co-cultivation with HT cells (later with clone H4) was performed on December 29, 1983. Unusually high numbers of multinucleated giant cells were observed seven (7) days after co-cultivation. Using ET serum and later the rabbit anti-HTLV-III antibody, a high percentage of positive cells was found in IF assays with characteristic membrane fluorescence. The percentage of IF positive cells was in the range 33% to 80%. Reverse transcriptase (RT) activity was consistently positive in culture fluids harvested from H4/RF cultures and values fluctuated in the range from 2.5×10^3 to 10^5 cpm with rAdT and about 10^3 cpm with dAdT. On December 29, 1983, E. Read sent W7152 for EM examination to Dr. M. Gonda (FCRF) and results obtained on January 18, 1984 indicated that the cell samples are negative for HTLV-III (see enclosed EM pictures). Because of consistent positivity in both IF and RT assays, we twice sent H4/HTLV-III$_{RF}$ cells for EM examination, namely on January 19 and March 13, 1984. The results again indicated that the cell cultures were negative for any HTLV-I, -II or -III type viruses (see letter of March 26, 1984 and EM pictures).

In the meantime, the HTLV-III$_{RF}$ isolate was cloned and a restriction map of the provirus genome clearly demonstrated that this is the most divergent type HTLV-III isolate compared to HTLV-III$_B$, MN, etc.

I requested from Dr. M. Gonda reexamination of the original blocks of H4/HTLV-III$_{RF}$ cells again in EM and, at this time, more careful analysis showed that the H4/HTLV-III$_{RF}$ cells were positive for HTLV-III particles (see enclosed letter of October 17, 1984 and EM).

The strategy we used to prepare a productively infected HT cells for the large scale production of HTLV-III was as follows:

In the first approach we infected H9 cells with a pooled and concentracted supernatant (positive in RT assays) harvested from different patients with AIDS and pre-AIDS. HT cells (clone H9) were repeatedly exposed to such concentrated culture fluids in order to maximize the percentage of HTLV-III positive cells.

In the second approach we attempted to obtain a single isolate from a patient who is well documented (sufficient amount of sera and primary non-activated lymphocytes would be available, e.g., RF).

Because of the lack of EM evidence in the case of HTLV-III$_{RF}$ isolate we decided to pursue the isolate(s) obtained from pooled culture fluids known as HTLV-III$_B$. Because of enormous requests and pressure from other laboratories to provide HTLV-III, we disseminated H9/HTLV-III$_B$ into laboratories all around the world before all control assays were completed. Fortunately, extensive analyses of H9 cells performed in collaboration with Dr. J. Lemp (Electronucleonics, Silver Spring, MD) showed that H9 cells are negative for any viruses or PPLO and H9/HTLV-III$_B$ is harboring only AIDS retrovirus (HTLV-III).

At the present time we are intensively pursuing the single-cell cloning and superinfection of H9 cells and other target cells to achieve 80% to 90% positivity for HTLV-III$_{RF}$. This work should be accomplished within a few weeks, and then HTLV-III$_{RF}$ together with patients' serum will be distributed in some selected laboratories according to importance of work and requests.

Mikulas Popovic, M.D.

Encls.

xc:
Dr. P. Fischinger
Dr. W. Blattner
Dr. S. Broder
Dr. M. Sarngadharan
Dr. F. Wong-Staal
Dr. J. Hoxie
Ms. E. Read-Connole