The University of Alabama at Birmingham
Division of Hematology/Oncology/Department of Medicine

April 6, 1990

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National Institutes of Health
9000 Rockville Pike
Building 37, Room 6A
Bethesda, MD 20892

Dear Bob:

It was nice to see you in Keystone! After our discussion, I carefully searched through all our laboratory notebooks to provide you with answers to some of the questions raised in the context of the inquiry. To make things easier, I will first address specific questions and then provide you with a detailed time table that relates to studies done by George and myself. As you can see from the photocopies, George's notebooks are excellent with respect to detail and definitive dates. Unfortunately, my notebooks are much worse. However, taken both George's and my notebooks together, I was able to date the most important experiments and determine a time table.

I-3. **When was HTLV-IIIb first identified?**

The first mention of H9/HTLV-IIIb is in George's notebook (dated 4/17/84), however, there is no mention when the DNA was extracted and where it came from. In this experiment, H9/HTLV-IIIb was first analyzed by Southern Blot analysis together with MOV and normal thymus DNA, and was probed with HTLV-I probes since we did not have any HIV-1 specific probe at that time. H9/HTLV-IIIb was analyzed again 4/24/84 as well as 4/26/84. All these filters were hybridized with subgenomic HTLV-I probes under high and low stringencies. Finally on 4/29/84 a Southern Blot comparison was made between MOV, H9/HTLV-IIIb and normal thymus using cDNA preparations from Sasha for probe. MOV and H9/HTLV-IIIb were clearly positive for HIV-1 in this analysis (I have sent you a picture of this particular blot). On 5/17/84 George started to construct the XbaI library from H9/HTLV-IIIb DNA which subsequently lead to the identification of lambda HXB2 and lambda HXB3.

Was a comparison made between HTLV-IIIb and LAV? When?

After having cloned H9/HTLV-IIIb, we used the BH10 probe to compare by Southern blot analysis H9/HTLV-IIIb, and LAV-B. These analyses were done late August 1984 and beginning of September 1984 in several different experiments, and they are recorded in my notebook under experiment B_6 and E_6. Experiment E_6 is dated 9/2/84. The analysis showed restriction enzyme identity between H9/HTLV-IIIb and LAV-B, however, the viral load in the LAV-B cell line was about 1/10th of that in H9/HTLV-IIIb. Because the bands were so faint, we actually hybridized LAV-B under normal and relaxed stringency

conditions (experiment E_4). The final Southern Blot comparison between H9/HTLV-IIIb and CEM/LAV was made on 11/27/84 after I returned from Paris (Thanksgiving meeting at the Pasteur Institute). J.C. Cherman had given me high molecular weight DNA for this comparison.

II-2. **Did you have isolates distinct from HTLV-IIIb when the four Science articles were published in 1984?**

The first molecular evidence of independent isolates was obtained on 6/5/84 by comparing H9/HTLV-IIIb to W7152 (patient RF). This blot shows clearly the genetic diversity between these two strains.

III-1. **When did you receive the French virus LAV? Is receipt of the virus referenced in any of the laboratory notebooks?**

The first reference to "a French virus" in my notebook is around Easter 1983 (Experiment O_4). In this experiment JBB/LAV was compared with MT2, normal thymus and MOV DNA. The blot was probed with HTLV-I and HTLV-II probe and JBB/LAV was negative with both probes. However, MOV DNA was clearly strongly positive using the HTLV-II probe.

There also is an experiment done by Anita Lomonico on 4/29/83 which includes "two French AIDS samples" (designated A31 and A32), which are compared to Hut102, normal thymus and MOV DNA. Again HTLV-I and HTLV-II probes were used in this analysis and the AIDS samples were negative. However, MOV was found to be infected with HTLV-II virus. I have no records determining what A31 and A32 was.

IV-1. **For what purpose was the designation of "MOV" used in LTCB?**

Going through George's and my notebooks there are clearly two different preparations of "MOV" that were analyzed. The earliest mention of "MOV" is in early April 1983. There are several experiments where MOV is compared to Hut102 as well as several "AIDS" samples (designated A10, A11, A33b, A35, Cocult Chardon, etc.). This preparation of MOV was clearly infected with an HTLV-II type virus. It was never retrospectively analyzed using an HIV-1 specific probe. The pertinent experiments are in my notebook (called Southern blot book I) and they include experiments Y_3 and Z_3.

The second preparation of MOV DNA is first noted in George's notebook at 4/13/84. This DNA preparation was subsequently analyzed for HIV-1 sequences and is identical in restriction pattern to H9/HTLV-IIIb. From these experiments it is apparent, that MOV at one point in time was infected with an HTLV-II type virus. A year later when the DNAs were re-extracted it is clearly infected with HIV-1. The 1983 sample was never probed

with HIV-1, so it is not clear whether it was infected with both viruses at that time. The 1984 preparation was never probed with HTLV-II, so it is again not clear whether at that point in time MOV was still infected with an HTLV-II type virus.

13. **Questions about HTLV-IB.**

Work on Elomata and McCarey was done independently by myself and George. George's records show that he started cloning McCarey immediately after his arrival in August 1983. The code number for McCarey was W3774. The code number for Elomata was W3734. George started cloning of McCarey in 9/26/83 and molecular clones were available at 10/16/83. Restriction enzyme analysis at that point showed that the McCarey clones were quite distinct from prototypic HTLV-I strains, and the conclusion was drawn that this virus was a variant of HTLV-I possibly causing AIDS in the patient McCarey. Subsequently, George wrote a paper that was submitted to Science, but then rejected because it only described a single patient. While studies were ongoing with cloning and analyzing McCarey, I worked on the African ATL culture termed Elomata (W3734). Around the middle of January 1984, we discovered that clones derived from Elomata and McCarey were identical in their restriction enzyme pattern. A site-by-site comparison using 14 restriction enzymes demonstrated identity of clone MC-1 and clone E-3K (the digest is in my cloning book II). Based on this information, we realized that a mistake had to have occurred and we proceeded to identify which of the two cell lines (both grown in Mika's lab) corresponded to which patient. We asked Dean Mann to determine the HLA types on both the McCarey and the Elomata cell lines and Dean's analysis proved that both cell lines were the same. Moreover, Dean's analysis also identified an independent Elomata culture as having the same HLA type. Comparing the code numbers W3774 for McCarey and W3734 for Elomata, it became apparent that a mislabelling had occurred in Mika's lab. After this mistake was cleared up, we proceeded to publish the real African HTLV-Ib in the International Journal of Cancer.

TIMETABLE

4/29/83	Anita Lomonico (while still working with Ed Gelman) compares two "French AIDS samples" with Hut102, normal thymus and MOV DNA using HTLV-I and HTLV-II probes. The analysis identifies MOV to be infected with HTLV-II.
May or June 1983	The first French sample designate JBB/LAV is compared to MT2, normal thymus and MOV DNA using HTLV-I and HTLV-II probes. Again, the French samples are not infected with HTLV-I or HTLV-II, however, MOV is clearly identified as containing an HTLV-II type virus. The French

sample, designated JBB/LAV, was never retrospectively analyzed with an HIV-1 probe.

8/15/83 — High molecular weight DNA is extracted from W3774 (McCarey) and C103+W3731 (Chardon).

9/21/83 — Chardon library is being constructed

10/1/83 — McCarey clones are identified. Chardon clones are being mapped in detail.

10/19/83 — DNAs are obtained from sample W3771 (J.P., a LAS patient from Kaplan, cultured by Mika).

1/4/84 — HTLV-II is cloned from JP.

1/26/84 — Discovery of restriction map identity between clones from McCarey and Elomata.

3/7/84 — First cloning attempt of LAV. George constructs a MboI partial library of "LAV" DNA (probably obtained from Mika). Library is being screened with HTLV-I and HTLV-II subgenomic probes under low stringency. Clones are identified, however, they contain normal human sequences related to HTLV-I and HTLV-II and not the AIDS retrovirus.

4/13/84 — New extraction of MOV DNA (presumably from Mika).

4/17/84 — First mention of H9/HTLV-IIIb

4/29/84 — Comparison of MOV, H9/HTLV-IIIb, and normal thymus using cDNA probe from Sasha. The blots demonstrate the presence of HIV-1 in MOV and H9/HTLV-IIIb.

5/2/84 — Successful Hirt preparation for the cloning of HIV-1 done in collaboration with Mika. The source of virus for the Hirt preparation is H9/HTLV-IIIb (Electronucleonics). This is the preparation from which BH10, BH8 and BH5 are being cloned.

5/28/84 — The same cDNA probe is used to characterize several of Mika's independent isolates. JK, JR, LS, MR, LW, mROD all exhibit the identical restriction enzyme pattern as compared to H9/HTLV-IIIb.

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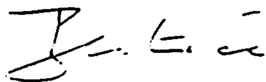
5/29/84	An XbaI library is constructed from H9/HTLV-IIIb DNA.
6/5/84	W7152 (RF) is identified as an independent and genetically distinct isolate.
6/9/84	HXB2 and HXB3 are being isolated from the H9/HTLV-IIIb library.
7/14/84	Molecular cloning of W7152 (RF)
9/2/84	Comparison of H9/HTLV-IIIb to LAV-B obtained from Mika.
9/28/84	HIV-1 is detected in brain samples from ADC patients.
11/27/84	Comparison of CEM/LAV and H9/HTLV-IIIb identifies restriction enzyme site identity.
1/27/85	Several DNA samples are obtained from Robin Weiss. Southern blot analysis identifies restriction enzyme cleavage pattern identity among all five samples including H9/HTLV-IIIb.

Please let me know if you have additional questions. I am enclosing photocopies of all the pertinent parts of the notebooks so you can find them in the original. Good luck.

Sincerely,



Beatrice H. Hahn, M.D.
Associate Professor
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BHH/ajn

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1. DNA extraction from MOV 4-13-84
2. first mention of H9/HTLV-IIIb 4-17-84
3. cloning of H9/HTLV-IIIb 5-17-84
(→ λHXB2 and λHXB3)

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New Kit preparation   MiPa 8×10^9

40C particles / cells

5/2/84

target cells : H9

infectivity: 4×10^{11} particles ~ 4×10^8 IPU.

amount of cells: 1.5 gr per timepoint (x5)   7.5 gr   1 gr = 10^9

time points :

infection time   5/2/84   7:30 pm

1. harvest   12:30 am   15 + 15 + 6

2. harvest   7 am   20 + 20 + 8

3. harvest   10:30 am   20 + 20 + 8

4 harvest

5. harvest

Source: H9 - HTLV-III B (Electronucleonics)

4h	2.0 mg	digest 30 μg in 100 λ	
10.5h	2.69 mg	1	∅
15h	2.88 mg	2	B_1
24h	4.24 mg	3	SstI
		4	HindIII
		5	BamHI
		6	XbaI

1st $CsCl_2$ gradient   4 gr CsCl + 4 ml Kit + 40λ EtBr.

μ = 1.394

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RUB DNAs

A31 Code : 3 RUB DNA from 3 days old cultured lymphocyte

350 ug/ml sample : 35 ug/100 ml

A32 Code : 4 RUB DNA from RUB lymph node

1250 ug/ml sample : 75 ug

Buffer : Tris 10 mM pH 7,5
EDTA 1 mM

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